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Abstract: We determined the effect of a GnRH vaccine on reproductive function of sexually mature 5-month female and male domestic swine. The vaccine, GonaCon™, developed at NWRC contains a GnRH peptide conjugated to KLH, combined with AdjuVac™ adjuvant also developed at NWRC. Four groups of ten females were given single IM immunizations either of 800µg GnRH vaccine, 1600µg GnRH vaccine, a prime and boost of 400µg GnRH vaccine and a sham dose adjuvant alone (control). At 8 months old, females were evaluated daily for estrus and bred by artificial insemination if in standing heat. Females not showing heat after 60 days were considered infertile. Farrowing data were collected for bred females ~115days later. All control gilts showed heat and became pregnant, whereas none given the dual 400µg dose showed heat or were bred. Of the 10 gilts receiving 800µg GnRH vaccine 3 were bred, but only 2 became pregnant. For gilts receiving 1600µg, 1 showed heat and became pregnant. Serum antibody titers were non-detectable in the control group, highest in gilts receiving 2×-400µg, followed by 1600µg and 800µg treatments. Titers were inversely correlated to suppression of estrus and fertility. Groups of five boars received either adjuvant only, 1×- 800µg or 1600µg, or 2×- 400µg GnRH vaccine IM. Blood and scrotal testicular measurements were taken initially and 4 and 16week post-treatment. Testicular size and serum testosterone decreased in all groups except controls and was inversely related to serum antibody titers. The authors conclude that; injectable GnRH vaccine is effective for contraception of swine.

Key words: domestic swine, gonadotropin releasing hormone, immunocontraception, single shot

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INTRODUCTION

The population of feral swine in the U.S. now exceeds 2 million animals. At least 26 states now have feral swine populations. Endemic in most of these populations are the diseases pseudorabies and brucellosis. With the near eradication of both of these diseases in domestic swine, their presence in feral swine places at risk the local domestic populations and has become an important issue. Research needed to address the feral swine disease issues includes development of safe, more efficacious oral vaccines and vaccine delivery systems as well as the development of more effective population control methods. The development of an efficacious, administered immuno-contraceptive vaccine for feral swine would

be a valuable tool for use in disease management of pseudorabies and brucellosis (Rhyan and Drew 2002).

As part of the program to develop contraceptive tools to control populations of over-abundant wildlife species, the NWRC has researched the PZP and the GnRH contraceptive vaccines (Miller and Fagerstone 2000). We found that PZP in white-tailed deer effectively contracepted the deer, however the deer experienced multiple estrus cycles many times into February (Miller et al. 1999, Killian and Miller 2000) Our first study using the GnRH vaccine was in the Norway rat (Miller et al. 1997). The rats remained contracepted throughout their short life. We then tested the GnRH vaccine in white-tailed deer with good success. (Miller et al. 2000). We are currently testing a newly developed single shot vaccine in white-tailed deer with good success.

Immunization against the GnRH hormone prevents the circulating GnRH from stimulating the release of pituitary LH and FSH. In order to successfully neutralize GnRH, high titers of specific anti-GnRH antibody must be present to complex with the hormone and prevent it from binding to pituitary receptors. Immuno-neutralization of GnRH produces a temporary non-surgical castration in animals (Meloen et al. 1994, Oonk et al. 1998).

To determine if a single-shot GnRH vaccine would be effective for contraception of feral swine, we used domestic swine to determine vaccine dosage and duration of the contraceptive effect. Zeng has recently tested a vaccine design in Chinese male pigs (Zeng et al. 2002).

THE SINGLE SHOT VACCINE

Development of immunocontraceptives that are practical to use for wildlife population control must consider how the vaccine can be delivered. While administration of an oral form of the vaccine may be the ultimate goal, a long acting single-shot injectable form of the vaccine would also have practical advantages over the prime and boost vaccines currently in use.

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 which may last for months to years. Literature reports on antigen structure and retention have guided our efforts to produce a vaccine that is effective with one injection. (Burton et al. 1994) have pointed out that the long-lasting immune response needed for immunocontraception is accomplished with "retained antigen." Without a continued release of antigen (in this case KLH-GnRH), the immune response will decrease and the contraceptive effect with cease. Therefore, the antigen should remain in the host for a sufficient period so that immune-complexes are formed from antigen and specific antibody stimulated by the vaccine. In the boost response, specific antibody is rapidly produced and binds with antigen producing immune-complexes (IC). The immune complexes (IC) formed from the combination of antigen and antibody are transported into the draining lymph node where the vast majority of the IC's are eliminated by phagocytic cells. However, a small portion of complexes are trapped on the surface of follicular dendritic cells (FDC) in the micro environment of the draining lymph node.

The FDC-mediated pathway of antigen handling is responsible for the induction and maintenance of long-term antibody response. FDCs trap ICs and retain picogram amounts of antigen contained in the lymph nodes for long periods of time,

making intact antigen available to the immune system for months to years. Binding of the antigen to the antibody protects the conformation of the antigen, ensuring continued presence of the original antigen conformation.

Optimal vaccine design should create an immunogen that mimics pathogenic bacteria. Many pathogens, including viruses and bacteria, exhibit rigid, highly organized, highly repetitive protein epitopes. This repetitive epitope pattern provides a cross-linking activation of B cell receptors providing an extremely strong long-lasting immune response (Bachman et al. 1993, Bachman and Zindernagel 1996). This mimic of the repetitive nature of pathogen epitopes was an important part the KLH-GnRH conjugate design for our vaccine. Banatvala et al. (2001) proposed that the antigen dose and structure influence the magnitude of the primary response. The greater the primary response, the longer the duration of the immune memory. To have success with a single injection, the dose and the timing of the injection is more critical than that of a two shot.

We have observed that the adjuvant portion of the vaccine is critical to the success of the vaccine (Cooper 1994). The new KLH/GnRH vaccine we developed uses an adjuvant developed at the NWRC containing a modified USDA-approved Johne's vaccine, Mycopar™, as a replacement for Freund's adjuvant. Mycopar is approved for use in food animals and should not be a concern for use in deer or pigs, which may be eaten by hunters. The new adjuvant, which we have labeled AdjuVac™, contains a small quantity of *Mycobacterium avium* bacteria to which wildlife and domestic animals are commonly exposed.

This paper reports on immunocontraception of both female and male domestic pigs using the newly developed, single shot GnRH vaccine which has a USDA, APHIS patent pending status.

METHODS

Female Pigs

Forty 5-month-old gilts, held at the swine facility at Penn State University, were divided into groups of 10 each. The groups were given doses of KLH/GnRH/AdjuVac vaccine as follows:

1. GnRH (800µg) prime only
2. GnRH (1600µg) prime only
3. GnRH (400µg/400µg) prime and boost
4. Control AdjuVac only

Groups were bled and given the primary vaccination in late October 2001. All groups were again bled and Group 3 boosted in December. During January/ February gilts were bled once and teased daily with a boar. Gilts that showed heat were bred by artificial insemination (AI). Blood serum was collected and tested for anti-GnRH by ELISA and progesterone by RIA. Infertile gilts (not showing heat) were put on pasture in March.

Male Pigs

Four groups of 5 adult male pigs were given doses of KLH/GnRH/AdjuVac vaccine as follows:

1. IM GnRH (800µg) prime only
2. IM GnRH (1600µg) prime only
3. IM GnRH (400µg/400µg) prime and boost.
4. Control

All pigs were pre-bled prior to vaccination and primed in May. All groups were bled in June and Group 3 was given a boost injection. Serum collected during the bleeds was tested for anti-GnRH titers by ELISA and testosterone by RIA. Body weights were measured throughout the study and testis and epididymis weights were taken at slaughter.

RESULTS

All groups of treated females and males produced an antibody response to the GnRH vaccine in a dose-related manner. Tables 1 and 4 show the means and ranges of antibody titers in the female and male groups. The immune response of females was greater than that observed for the males. For the 800µg single dose, the mean antibody response was 28k in the male and 70k in the female, and 80% of the females were infertile (Tables 2 and 3). The maximal response of 100% contraception was seen with the two shot injection regimen using a 400µg prime and 400µg boost (Table 2). However, the 1600µg single shot contracepted 90% of the females, indicating the response of the single shot vaccine can approach the maximum response seen with the two shot.

Table 1. Mean and range of GnRH antibody at the January breeding for females.

	Mean x 1000	Range
Control	0	0
800µg	70	16-128
1600µg	134	32-256
400/µg 400µg	208	64-256

Table 2. Percentage contraception in each group of females.

Control	0
800µg	80
1600µg	90
400µg/400µg	100

Table 3. Farrowing data for females.

Control	n =10	105 pigs/10.5 pigs/litter
800µg	n =2	19 pigs/9.5 pigs/litter
1600µg	n =1	5 pigs/5 pigs/litter
400µg/400µg	n =0	0 pigs

Those gilts not pregnant were pastured for re-evaluation in a second breeding

Table 4. Mean and range of GnRH antibody at 3.5 months after vaccination for males.

	Mean x1000	Range x1000
Control	0	0
800µg	28	16-128
1600µg	130	32-128
400µg/400µg	102	32-128

Table 5. Mean testosterone of males at the start of the study and after 3.5 months.

	Start µg/100ml	3.5 months µg/100ml
Control	147	202
800µg	180	162
1600µg	185	167
400/µg/400µg	208	52

Table 6. Mean testis weight (grams) of males at 3.5 months. L=Left, R=Right.

Control	L 421 R 398
800µg	L 291 R 318
1600µg	L 247 R 256
400µg/400µg	L 177 R 194

Table 7. Mean body weight (lb) of males at start of study and at 3.5 months.

	Start	3.5 months
Control	272	354
800µg	254	352
1600µg	269	353
400µg/400µg	256	345

Litter size of the control pigs averaged 10.5 pigs (Table 3), which was greater than that seen in the 800µg and 1600µg treated females, averaging 9.5 pigs and 5 pigs per litter, respectively. Because the two-shot dose was 100% effective, there were no litters produced. Although with only 1 litter produced in the group receiving the 1600 dose it is difficult to come to a definitive conclusion, it is possible that partial GnRH contraception may have reduced litter size. A reduction in litter size is possible if high titers of GnRH antibody reduce the number of multiple ovulations characteristic of swine. Alternatively, if ovulation does occur, GnRH antibodies could reduce GnRH stimulation of LH secretion for normal corpus luteum maintenance. This in turn may result in lower progesterone to maintain pregnancies with normal numbers of embryos.

In the control male pigs, testosterone increased with age (Table 5). The two single-shot pig groups demonstrate a consistent reduction in testosterone by the end of the study, compared to the increased testosterone with increased age observed in the controls. The reduction in testosterone in the 2-shot group is striking. Testicular size (Table 6) and serum testosterone decreased in all groups except controls and was inversely related to serum antibody titers.

There was no affect of treatment on weight gain throughout the study among the control and treated groups (Table 7). Average body weights of all groups increased by 34%, regardless of treatment.

DISCUSSION

The NWRC initiated research on the GnRH vaccine because immuno-contraceptive porcine zona pellucida (PZP) vaccine used for deer produced a side effect of repeated estrous cycles (Miller et al. 1999). A practical concern was that large scale field use of the PZP vaccine would increase car-deer collisions as a result of increased breeding activity and an extended breeding season. Most previously-tested GnRH vaccines have reported a short-lasting immune response and required multiple injections to provide a long-lasting immune response. Because the characteristics of these GnRH vaccines made them impractical to use for the contraception of wildlife, we began research to develop a single-shot GnRH vaccine with an adequate immune response.

In addition to pigs, this vaccine has been tested in rodents, deer, bison, wild horses, cats and dogs. In each case the vaccine proved effective in reducing concentrations of sexual hormones and reproductive activity of the injected animal for at least one to two years with a single injection and several years with 2 injections. Contraception with the GnRH vaccine in females has been more effective than that found in males. This is likely related to the differences in GnRH and gonadotropin secretion patterns (cyclic versus steady) between females and males. If the monthly LH spike in the female is reduced, and infertility has been induced. The constant LH demand in the male produces a greater demand on the anti-GnRH antibody. It also may be easier to shut down a seasonal animal such as the deer, compared to the pig which can breed year around.

This single shot GnRH vaccine was first tested in white-tailed deer at the Pennsylvania State University Deer Research Center. During the first year, the vaccine induced 100% infertility in both males and females, totally shutting down testosterone to a non-detectable level in all males tested. In this study GnRH vaccine in the female demonstrated a dose relationship in number of heats experienced and farrowing rates, with the 800µg and 1600µg achieving an 80% and 90% reduction in

fertility, respectively. However, only the 2 shot dose achieved 100% reduction in fertility. These data show the importance in testing the effectiveness of immunocontraceptive vaccines in each target species.

The effectiveness of the GnRH vaccine in deer and swine was different. Deer were injected in July while in a sexually dormant condition. The GnRH vaccine induced an antibody titer that was present prior to and during the fall season when endogenous GnRH production was peaked. The GnRH antibodies present prevented both sexes from developing the normal fall rut activity. In the female pig, hormonal activity fluctuates with the estrous cycle (Schneider et al. 1998, Zeng et al. 2002). Immuno-inactivation of GnRH activity prevents stimulation of the LH pulse and inhibits heat and conception. In contrast, the male pig has a non-pulsatile secretion of GnRH and a relatively steady secretion pattern of testosterone. Consequently, because GnRH secretion is relatively constant in the male, it is likely that higher titers of GnRH antibody are required to inactivate endogenous GnRH.

A second possibility for the higher vaccine dose required for female and male pigs is that some of the vaccine injected may be absorbed in the pig fat. Dr. Robert Brown from ImmunoVaccine Technologies (personal communication) has experienced this when injecting seals and possibly also in black bears. Vaccine trapped in fat may not be released to the immune system, and therefore may be unavailable to induce an immune response.

SUMMARY

The single-shot GnRH vaccine reduced the fertility of female domestic pigs in a dose related manner. The vaccine also reduced plasma testosterone and testicular size of intact boars. The high-dose single-shot vaccine produced results similar to that of the 2-shot vaccine in the female. In boars, the single shot 800 µg and 1600 µg vaccines caused a drop in plasma testosterone by the end of the study, although the 2-shot vaccine resulted in the greatest drop in testosterone. The reduction in testis weight was correlated with the drop in testosterone. However, the single-shot vaccine was generally more effective in females than males.

We conclude that the GnRH vaccine has significant potential for use in limiting fertility of both males and females of many of domestic and wildlife species.

LITERATURE CITED:

BACHMANN, M.F., U.H. ROHRER, T.M. KUNDIG, K. BURKI, H. HENGARTNER, AND R.M. ZINKERNAGEL. 1993. The influence of antigen organization on B cell responsiveness. Science 262:1448-1451.

_____, AND R.M. ZINDERNAGEL. 1996. The influence of virus structure on antibody responses and virus serotype formation. Immunology Today 17:553-558.

BANATVALA, J., P. VAN DAMME, AND S. OEHEN. 2001. Lifelong protection against hepatitis B: the role of vaccine immunogenicity in immune memory. Vaccine 19:877-885.

BURTON, G.F., A.K. SZAKAL, Z.F. KAPASI, AND J.G. TEW. 1994. The generation and maintenance of antibody and B cell memory: the role of retained antigen and follicular dendritic cells. Pages 35-50 *in* G.L. Ada, editor. Strategies in vaccine design. R.C. Landes Company, Austin, TX, USA.

COOPER, R.D. 1994. The selective induction of different immune responses by vaccine adjuvants. Pages 125-158 i*n* G.L. Ada, editor. Strategies in vaccine design. R.C. Landes Company, Austin, TX, USA.

KILLIAN, G.J., AND L.A. MILLER. 2000. Behavioral observation and physiological implications for white-tailed deer treated with two different immunocontraceptives. Proceedings of the Wildlife Damage Management Conference 9:283-291.

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.

MILLER, L.A., AND K.A. FAGERSTONE. 2000. Induced infertility as a wildlife management tool. Proceedings, of the Vertebrate Pest Conference 9:160-168.

_____, 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. 1999. Long term effects of PZP immunization on reproduction in white-tailed deer. Vaccine 18:568-574.

_____, _____, AND _____. 2000. Immuno-contraception of white-tailed deer with GnRH vaccine. American Journal of Reproductive Immunology 44(5) 266-274.

_____, AND G.J. KILLIAN. 2000. Seven years of white-tailed immunocontraception research at Penn State University: a comparison of two vaccines. Proceedings of the Wildlife Damage Management Conference 9:60-69.

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 16:1074-1082.

RHYAN, J.C., AND M.D. DREW. 2002. Contraception: a possible means of decreasing transmission of brucellosis in bison. Pages 99-108 *in* T.J. Dreeger, editor. Brucellosis in elk and bison in the greater Yellowstone area. Greater Yellowstone Interagency Brucellosis Committee.

SCHNEIDER, F., H. FALKENBERG, G. KUHN, K. NURNBERG, C. REHFELDT, AND W. KANITZ. 1998. Effects of treating young boars with a GnRH depot formulation on endocrine functions, testis size, boar taint, carcass composition and muscular structure. Animal Reproduction Science 50: 69-73.

ZENG, X.Y., J.A. TURKSTRA, R.H. MELOEN, X.Y. LIU, F.Q. CHEN, W.M.M. SCHAAPER, H.B. OONK, D.A. GUO, AND D.F.M VANDEWIEL. 2002. Active immunization against gonadotropin-releasing hormone in Chinese male pigs: effects of dose on antibody titer, hormone levels and sexual development. Animal Reproduction Science 70: 223-233.