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Oral Vaccination of White-Tailed Deer Using a Recombinant Bacillus Calmette-Guérin Vaccine Expressing the *Borrelia burgdorferi* Outer Surface Protein A: Prospects for Immunocontraception

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PROBLEM: Reduction of excess numbers of white-tailed deer (*Odocoileus virginianus*) is a prime example of a potential use for immunocontraception as a means of wildlife population management. Oral vaccination appears to be the most pragmatic way to deliver immunocontraceptive vaccines to free-roaming populations of deer, but there was little, if any, prior evidence that oral vaccination is a viable concept in deer.

METHOD OF STUDY: We used live Bacillus Calmette-Guérin (BCG) in a recombinant form (rBCG), which expressed *Borrelia burgdorferi* outer surface protein A, to test whether deer vaccinated orally with a specific antigen expressed in a live vector produce detectable antibody titers.

RESULTS: The data indicate that oral vaccination of deer with an expressed antigen is feasible, as demonstrated by peak antibody titers to the expressed antigen. Also, peak titers measured by enzyme-linked immunosorbent assay were highest in orally vaccinated deer: 1600 in deer vaccinated by injection and 6400 in those vaccinated orally.

CONCLUSIONS: The results of this study demonstrate that it is feasible to vaccinate deer orally with a live vector.

Key words:
Bacillus Calmette-Guérin,
Odocoileus virginianus, rBCG

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INTRODUCTION

Reduction of excess numbers of white-tailed deer that generate human/animal conflict is a prime example of how immunocontraception may become a valuable wildlife management tool. In 1994, the population of this species in the USA was estimated at 15 million, the highest level this century.[1] Earlier attempts to limit deer reproduction with synthetic estrogens and progestins were generally ineffective or impractical for field use.[2–4] Recent research[5,6]

with similar hormones has led to improvement in efficacy of delivery and release, but they are still not practical for extensive management with large numbers of deer. Although immunocontraception has the potential to overcome some of the drawbacks inherent with earlier materials, effective delivery of immunocontraceptive vaccines to target populations remains a major hurdle in the development of this technology. Traditional vaccine delivery is by subcutaneous or intramuscular injection. Domestic pets and livestock, especially mammals, are easily and commonly vaccinated by injection. To achieve this form of delivery to free-roaming animals, the vaccine must be contained in a dart or a biobullet fired at individual animals, or the animals must be captured. Considerable success has been achieved via remote injection in a variety of species with the immunocontraceptive porcine zona pellucida (PZP).[7,8] However, while these methods used with PZP or similar vaccines may be workable with relatively small numbers of animals in certain confined locations, they are clearly unsuitable for vaccinating many animals in large-scale operations in a cost-effective manner. Conceptually, oral delivery represents a more practical alternative delivery method for most free-ranging wild animals. However, there are many problems associated with this approach that remain to be solved. Baiting of wildlife with a high percent success can be difficult; environmental stability of baits containing a vaccine is required, and oral vaccines for mammals have not been developed, except for polio and tuberculosis in humans and rabies in wildlife. There is little, if any, evidence to indicate that oral vaccination is a viable concept for deer, and whether an oral vaccine would survive the ruminant digestive tract. In mammals, oral immunization occurs via the mucosal immune system that primarily consists of the tonsils and other pharyngeal immune follicles, and small intestine containing Peyer's patches and other mucosal tissue.[9] If vaccination does not occur before the vaccine reaches the stomach, most vaccines will be digested and rendered inactive, unless they have been encapsulated or otherwise protected from stomach acid. However, some microorganisms are able to transit the stomach and remain intact (e.g., intestinal diseases like cholera and typhoid fever). Molecular biology and immunology have provided us with the technology to use live bacteria and viruses as vectors to carry vaccines. The most prominent example of this technology in wildlife management is the use of an attenuated live virus containing an inserted rabies gene to deliver oral rabies vaccines to raccoons and other wildlife.[10,11]

Our study represents a cooperative effort involving the National Wildlife Research Center (NWRC); the Pennsylvania State University (Penn State); and MedImmune, a biotechnology company in Gaithersburg, MD. The objective of the study was to determine whether a single oral vaccination using a live recombinant vector produces significant antibody titers in deer to a specific antigen.

MATERIALS AND METHODS

The vector used was a genetically engineered, live, attenuated, tubercle bacterium, recombinant Bacillus Calmette-Guérin (rBCG), provided by MedImmune. BCG was chosen as the vector for the antigen because of a good safety record and proven performance as an oral tuberculosis vaccine in its original nonrecombinant form.[12] It was first used to vaccinate humans in 1921, and has been used safely throughout the world to orally vaccinate over 2.5 billion people against tuberculosis.[13] It is currently being recommended by the US Centers for Disease Control for vaccination of health-care workers in high-risk settings.[12]

Recombinant forms of BCG (i.e. rBCG) have been used to deliver expressed antigens, serving as vaccines themselves, both systemically and orally.[14,15] Stover et al.[16] described the production of the recombinant used in this study. It contains a DNA insert that encodes for the antigen outer surface protein A (OspA), a protein found in the spirochete *Borrelia burgdorferi*, the infective organism of Lyme disease. The OspA antigen is expressed on the exterior of the rBCG bacterium. This position enhances the effectiveness of the delivery of the antigen when taken into the mucosal immune system of the vaccinated individual. The specific concept and process by which a live oral vector is used to deliver a protein antigen produced from an inserted segment of DNA, consists of many important elements that are beyond the scope of this paper. The interested reader is encouraged to refer to the review presented by O'Hagen[17] for further information. The use of OspA as the specific immunogen had no special significance other than as a model antigen to assess the immune response, to the oral vaccine.

Ten white-tailed deer (3 male and 7 female, aged 1–2 years) were housed at Penn State in 3.0- by 9.1-m chainlink pens on concrete floors in a facility rated at Biosafety Level 2[18] with practices, animal care, and containment equipment appropriate for that level. Prior to rBCG exposure during the study, and at the conclusion of the study, each animal was evaluated for the presence of bovine tuberculosis by the Tuberculin™ PPD Bovis, Intradermic skin test. The num-

bers and sex of the deer on each treatment were selected, with sample size emphasis (4 vs. 2) on the oral group; the deer were then assigned randomly to treatment groups as follows:

- Group A (1 male, 1 female): controls (OspA), primed subcutaneously with 500 μg OspA alone and boosted 21 days later with 300 μg to determine whether the deer would mount immune responses against the protein;
- Group B (1 male, 1 female): controls (BCG), primed subcutaneously with 10^8 organisms using a nonrecombinant BCG to determine control immune responses;
- Group C (2 females): injection delivery (rBCG), primed subcutaneously with 10^8 organisms of the rBCG to provide comparative immune responses to oral delivery.
- Group D (1 male, 3 females): oral delivery (rBCG), primed orally with 10^9 organisms of rBCG suspended in 20 mL of a 5% sodium bicarbonate isotonic saline solution to reduce stomach acidity. The dose was administered as an oral cavity lavage via a 50-mL syringe sans needle. The number of organisms given orally vs. by injection, was increased from 10^8 to 10^9 because oral immunization is known to require higher doses than systemic immunization.[19] This is due partially to the limited absorption from the intestinal lumen of antigenic material that escapes digestive enzymes or complexing with preexisting antibodies and other substances such as mucin.

For animals receiving injections, the doses were contained in buffered saline resulting in a vaccine volume of 1 mL that was distributed among 8–11 injection sites in the scapular region in the center of the back with a 3-mL syringe and 22-gauge needle. This injection procedure minimizes the reaction to the vaccine while maximizing the immune response. Groups B, C, and D were boosted 4.5 months later with the same dose as the original vaccination. These groups were not boosted before 4.5 months because we anticipated that the BCG and rBCG organisms would remain viable in the animals and provide a boosting effect. However, this did not occur, as demonstrated by periodic blood sample examination; hence, a boost was given to determine its effectiveness.

Although it would have provided additional information to add a fifth group as oral controls that were orally dosed with nonrecombinant BCG, the size of the biosafety facility did not allow for additional deer. Therefore, the injected rBCG group C was considered a positive control for the rBCG antibody response.

To test for cross-infectivity of the oral rBCG, the two deer in control group A were housed between animals from oral group D; two group D deer were in each of the adjacent pens with a common chainlink wall covered with canvas between pens to prevent visual contact and reduce excitability of the animals. There was ample opportunity for cross-infection to occur via pen bedding moving between pens on the floor, aerosols, and general handling procedures. Oral and rectal fecal swabs, and pen bedding samples from these six deer were collected predose and at periodic intervals during a 75-day examination period after the first oral vaccination. The samples were analyzed for the presence of tubercle bacteria using a modified auramine O Richards acid-fast fluorescent staining procedure[20] and microscopic examination with a 490-nm excitation filter and a 520-nm barrier filter at magnifications of 200–1000. BCG acid-fast bacteria were distinguished from other tubercle bacteria by their lack of association with mucous material, and an overall appearance that suggested a slightly larger and more turgid shape. Fecal swab samples were sent to a contract laboratory (National Veterinary Services Laboratory, USDA/APHIS/VS, Ames, IA) for tubercle bacteria identification by culture. Venous blood samples were collected from each animal prior to the first vaccination and periodically throughout the study. Serum antibody titers were determined by enzyme-linked immunosorbant assay (ELISA) analyses performed at MedImmune. ELISA plates were coated with 100 ng of BCG Pasteur or OspA protein in carbonate buffer. The plates were blocked with phosphate-buffered saline-Tween 0.01%, 1% milk, 0.1% bovine serum albumin. The deer serum was serially diluted in blocking solution and added to the wells. Bound antibody was detected using rabbit anti-deer followed by goat anti-rabbit-HRP.

At the conclusion of the study, all deer were killed by injection of euthanasia solution and the carcasses were incinerated. All animal procedures used in this study were approved by the NWRC and Penn State Animal Care and Use Committees.

RESULTS

Passage of Recombinant Bacillus Calmette-Guérin

No oral shedding of the bacteria was detected after initial ingestion (Table I). Fecal shedding peaked at 24 hr (4/4 positive) but declined at 72 hr (2/4 positive). No acid-fast bacteria characteristic of the orally dosed rBCG were observed in the fecal swabs after 72 hr, or in random samples from the confinement pens for up to 91 days. An acid-fast bacillus trapped in mucous material was detected in fecal swabs from five of the six deer before treatment at the beginning of the study and in all six deer for 91 days during the study.

After the start of the study, it was determined that an avian tuberculosis infection was endemic in the Penn State deer herd. We assumed that the periodic shedding of a mucus-bound acid-fast bacillus represented the avian species of tuberculosis. Chronic infections of avian tuberculosis can be present for prolonged periods with no clinical symptoms or apparent detriment to the animal.[21] The rBCG was not found in pretreatment fecal swabs. It was present in fecal swabs at 24 and 72 hr; it was not encapsulated in mucus, and presented a general appearance of a slightly larger size and more turgid shape than the avian tuberculosis bacillus, indicating an organism different from the avian form. Attempts by the National Veterinary Services Laboratory to culture fecal swabs for rBCG failed due to the normally expected problem of an overgrowth of fungus on the samples. One deer in the oral rBCG Group D died on day 76 of the study. Necropsy revealed a chronic avian tuberculosis presence, but the cause of death was not determined.

Tuberculin Skin Tests

Results of the pretreatment tuberculin skin tests were all negative (Table II). A positive result was defined as a 4-mm or greater increase in skin thickness 72 hr after an intradermal injection of purified bovine tuberculin protein. The test is positive only if bovine tuberculosis bacteria are present; it is not a test for antibodies to the bacteria. Two deer from oral group D became positive during the study and remained positive at the end of the study.

Bacillus Calmette-Guérin (BCG) and Recombinant BCG Antibody Titers to BCG

There was little antibody response, by either the injection or the oral route, to the initial BCG and rBCG doses (Fig. 1). Note that pretreatment BCG and rBCG titer data were not included in Fig. 1 because of a sample contamination problem. However, baseline titers for this analysis typically range from 200 to 400 in deer from the Penn State herd. After this study, it became evident that the presence of avian tuberculosis was endemic in the Penn State deer herd. Therefore, all rBCG titers we obtained from untreated deer were collected from deer already infected with the avian bacillus. There was a variable titer response after the boost was given.

Outer Surface Protein A Antibody Titers

The OspA control animals (group A) developed high antibody levels after the boost, with titers to the OspA ranging from 3200 to 6400. These levels were expected because of the known high immunogenicity of OspA. This group was an important positive control because a white-tailed deer immune response to OspA had not been reported in the literature, even though white-tailed deer are considered an important part of the etiology of Lyme disease. There were only slight rises in OspA antibody titers after the initial dose of the rBCG (Fig. 2), indicating that a single vaccination was insufficient. Therefore, a boost was given 4.5 months after the initial vaccination. Blood samples taken 2 weeks after the boost demonstrated increased antibody titers to OspA, with the highest levels appearing in the oral rBCG group D.

DISCUSSION

The advantages of rBCG as a vaccine delivery vehicle are its potential safety and effectiveness in delivery of the expressed antigen orally. Although BCG is safe for human vaccine use, it would need to be clearly proven that a rBCG was safe in deer. The effectiveness of this particular rBCG vector is likely enhanced because the OspA protein is expressed on the surface

TABLE I. Presence or Absence of Recombinant Bacillus Calmette-Guérin (rBCG) Bacilli in Oral[a] and Rectal Smears and Pen[b] Samples from White-Tailed Deer Orally Vaccinated with rBCG

		Days (hr) postdose – rectal				
Deer/sex	Treatment	0 (0)	1 (24)	3 (72)	5 (120)	75
430m	Group A, control, outer surface protein A (injected)	–	–	–	–	–
436f		–	–	–	–	–
427f	Group D rBCG (oral)	–	+	–	–	–
444m		–	+	–	–	–
460f		–	+	+	–	–
475f		–	+	+	–	–

[a] *All oral smears on groups A and D deer were negative at 0, 24, 72, and 120 hr.*
[b] *All pen samples were negative throughout the 75-day examination period.*

TABLE II. Bovine Tuberculin Skin Test Measuring Presence of Bovine Tuberculosis Bacteria in White-Tailed Deer

Deer/sex	Treatment	Increased skin thickness (mm)[a]		
		Prevaccination	Post 69 days	Post 199 days
430m	Group A, control, OspA (injected)	0.2	0.8	1.5
436f		0.3	0.8	0.3
452m	Group B, control, BCG (injected)	0.2	1.5	0.2
461f		0.2	1.8	0.7
405f	Group C rBCG (injected)	0.0	1.7	1.5
437f		0.3	1.0	3.4
427f	Group D rBCG (oral)	0.0	1.6	0.5
444m		2.0	4.8	4.4
460f		0.0	1.7	died
475f		0.0	1.9	5.2

[a] *An increase in skin thickness of ≥4 mm 72 hr post test injection is positive.*
BCG, Bacillus Calmette-Guérin; rBCG, recombinant Bacillus Calmette-Guérin; OspA, outer surface protein A.

of the bacterium, thereby efficiently presenting the antigen to the immune system of the animal. During the development and testing of live vaccines for wildlife applications, a significant concern is the potential for unintentional intraspecies or interspecies transmission of the vector, either by direct or indirect contact. The findings of this study indicate that transmission of rBCG to deer in close proximity did not happen. This reduces concern about the potential for unintential transmission of the live vector.

The presence of avian tuberculosis in all the test animals may have affected BCG titer results because an immune tolerance to acid-fast bacilli would preclude a stronger antibody response to BCG or rBCG.[21] The fact that two of the deer (group D) did not show an increased BCG titer in response to the rBCG booster lends credence to this possibility. A high immune response to OspA was developed, even though an oral tolerance to tubercle bacteria could be present, depressing the immune response to the BCG portion of rBCG. This is an important observation because field application of this type of vaccine would likely encounter similarly infected animals.

The main disadvantage of rBCG as a vector for vaccines in deer is the lack of available diagnostic tests to differentiate it from a bovine tuberculosis infection. The current ELISA antibody test and the tuberculin skin test used in the USA will give positive readings for both. The reaction in the bovine tuberculin skin test is specific for the presence of bovine tuberculosis antigen, not the antibodies formed against it.[22] Therefore, when rBCG vaccination results in a positive skin test response, it gives a false positive indication of current bovine tuberculosis infection, and not a positive antibody titer indicating prior exposure. A polymerase chain reaction, a procedure used to amplify specific fragments of DNA, could be developed to identify the DNA added to the rBCG, or an ELISA test could be designed for antibodies to the vaccine. The ELISA test approach is presented by Cirillo et al.[23] Either could be used to differentiate rBCG vaccine from bovine tuberculosis. Only two of the eight deer given BCG or rBCG vaccine developed positive skin tests, and these were only slightly positive. The positive skin tests in these two deer suggests that rBCG remain sequestered in lymph nodes and is still available to the immune system. Since the highest antibody titers to either BCG or OspA were in the orally-treated group, as were the positive skin tests, it suggests that oral vaccination with rBCG is superior to vaccination by injection.

CONCLUSIONS

In spite of the various problems encountered in the conduct of this study, the results clearly demonstrate that white-tailed deer can be vaccinated orally using a live vector. Since vaccination for the purpose of immunocontraception must be oral to facilitate broad use for management of overpopulated areas, this demonstration of the feasibility of oral vaccination is an encouraging step. Effective, orally delivered, immunocontraceptive management of deer is several years from implementation and numerous questions remain to be resolved before this technology will be available as an operational tool. For example, it is still unknown whether the immune response in the rBCG-vaccinated deer occurred in the pharyngeal tissue, the small intestine, or a combination of these sites; a determination of this is important to the development

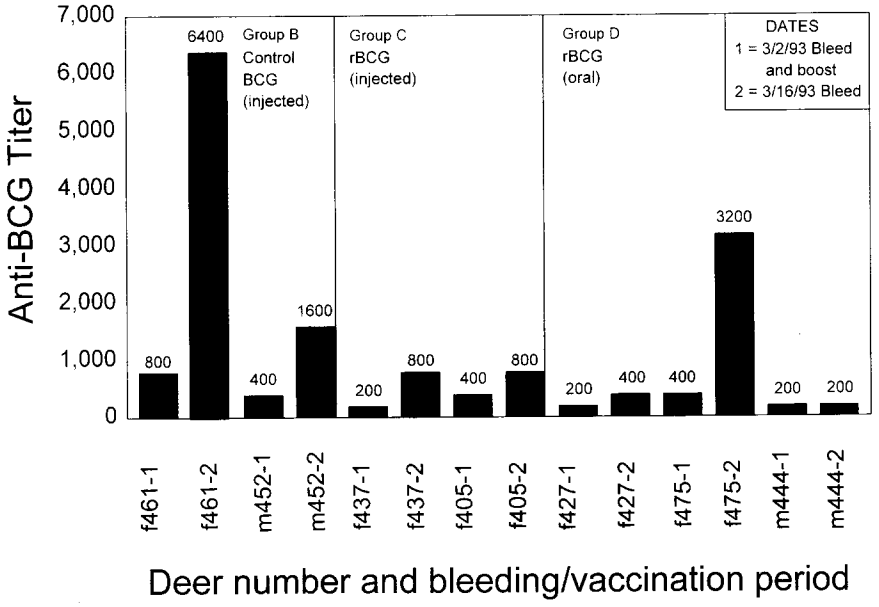

Fig. 1. Bacillus Calmette-Guérin (BCG) and recombinant BCG antibody titers (to BCG) in sera from white-tailed deer analyzed by enzyme-linked immunosorbent assay. All three groups received the prime vaccination 4.5 months before the boost vaccination.

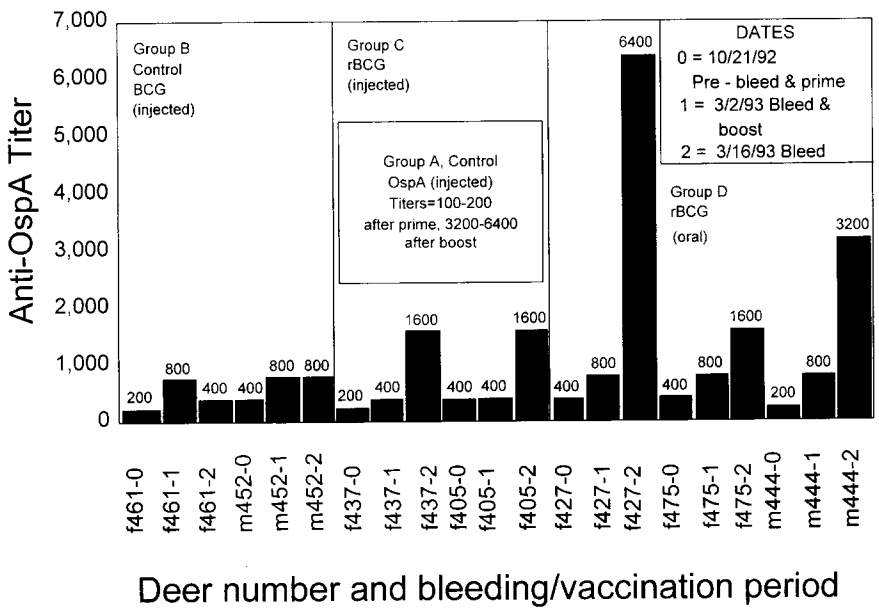

Fig. 2. Outer surface protein A antibody titers in sera from white-tailed deer analyzed by enzyme-linked immunosorbent assay.

of effective vaccine baits. Safety issues concerning the use of an oral, recombinate, live bacteria, or any other type of oral immunocontraceptive, must be resolved. The frequency and number of vaccinations must be reduced to a cost-effective level. Species-specific vaccines and delivery methods need to be examined. Additional future research will be needed to answer these and related questions. Nevertheless, it is likely that an oral immunocontraceptive vaccine will eventually become available as a new management tool to help solve problems of deer overpopulation in many parts of North America.

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