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**ABSTRACT:** Populations of wildlife, such as California ground squirrels, can grow to the extent that they come in conflict with humans. Contraception is a method of population management under investigation that may be useful in situations where neither leaving the animals uncontrolled nor lethal control are apropos. In this study, we tested the use of a single-injection gonadotropin releasing hormone (GnRH) immunocontraceptive vaccine in urban California ground squirrels. We monitored the effects of treatment for two breeding seasons. Immunization reduced the proportion of females lactating by 91% the first year and 96% in the second year. Testicular development was inhibited 35% the first year and 89% the second year. There is a delay of several months from the time of injection to inhibition of testes development. Reduction in the number of juveniles born per adult as determined by a visual count index was 9% the first year and 66% the second year. This study shows that the single-shot GnRH vaccine is over 90% effective for at least 1.5 years and requires several months after immunization for contraceptive effect. Because the immunization requires injection, it is labor intensive, but it is much more practical than treatments requiring multiple administrations to the same animal. GnRH immunocontraception may be a useful tool in rodent population management in certain circumstances.

**KEY WORDS:** California ground squirrel, contraception, fertility control, GnRH, gonadotropin releasing hormone, immunocontraception, population control, reproductive inhibition, *Spermophilus beecheyi*

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## INTRODUCTION

California ground squirrels (*Spermophilus beecheyi*) are a pest in agricultural and urban settings. They eat crops, dig burrows that can damage equipment and structures and cause personal injury, and are a potential vector for the spread of disease (Tomich 1982, Marsh 1994, Davis et al. 2002). In urban areas, the squirrels are often a source of controversy, with some people arguing for eliminating the squirrels and other people arguing for protecting them. Well-meaning people sometimes feed the squirrels, exacerbating the population problem and increasing problem behaviors. Management of squirrels can be made even more difficult by legal proscriptions on control methods.

Many of the problems associated with the squirrels would be of little consequence if populations could be kept at reasonable levels. Lethal control is the traditional method of population control, but in some situations killing the squirrels is not legal, practical, or publicly accepted. As contraceptive tools are developed for wildlife, they may become an alternative to poisoning for controlling populations and might be less controversial than current methods.

One category of contraceptive methods that holds promise for wildlife is immunocontraception. Immunocontraception involves immunizing an animal against some component of its own body that is necessary for reproduction. The immune system then either destroys part of the reproductive system or inhibits its function. There are a wide range of possible targets for such an approach, but most of the research that has been done has focused on the zona pellucida (the coating around the oocyte), sperm proteins, and gonadotropin releasing hormone (GnRH; a hormone that initiates the production of reproductive hormones) (see Fagerstone et al. 2002).

GnRH is formed in the hypothalamus and signals the pituitary to release two other hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH signal ovaries and testes to produce reproductive hormones and induce reproductive functions. Without GnRH, an individual would not develop sexually, and sexually mature individuals are infertile. Immunization against GnRH results in antibodies that bind GnRH and prevent it from signaling the pituitary. In the presence of sufficient antibody, the individual effectively has no available GnRH and the reproductive tissues do not function.

Immunocontraception with GnRH as a target has been tested in a variety of domestic and wild species, including cats (Ladd et al. 1994), dogs (Gonzalez et al. 1989, Ladd et al. 1994), pigs (Oonk et al. 1998, Dunshea et al. 2001, Miller et al. 2003), wild hogs (Killian et al. 2003), deer (Miller et al. 2000, Curtis et al. 2002), and rats (Awoniyi 1994, Miller et al. 1997). In each of these species, immunization leads to an inhibition of breeding behavior and contraception. Contraception by GnRH immunization continues as long as antibody titers remain sufficiently high. How long the antibody titer remains high enough is dependent on the species and on the vaccine formulation, with some variation between individuals.

Traditional formulations of immunocontraceptive vac-

cines require multiple injections over a period of time. For use in most wildlife species, this has been a major disadvantage. Recently, the development of a formulation that is effective with a single injection has rendered the concept of immunocontraception more practical (Miller et al. 2003). Animals must still be trapped for injection, but the necessity of recapturing the same animals within a certain time window for booster immunization is eliminated.

This study was undertaken at the Berkeley Marina, Berkeley, California, to test whether the single-shot GnRH immunization would be effective in wild California ground squirrels, and to get an indication of the feasibility of treating squirrels by injection. At the park, relocation sites were not available and poisoning had been prohibited. Squirrels in the park were numerous enough that they were becoming a hazard, and some method of control needed to be found.

## METHODS

### Study Area

The study was conducted in a park portion of the Berkeley Marina, covering about 5.1 hectares. The area contained a variety of terrain, including playground, rocks, beach, grass, and wooded hills. The squirrels had burrows in all these locations.

A site at Garretson Point was used as a control. This site covered about 1.7 hectares, with a similar variety of terrain.

### Experimental Time Course

Immunizations were initiated the end of August 2001 and continued through September 2002. The expected breeding season for California ground squirrels in this area is February and March. With a gestation period of 4 weeks, they would give birth in March or April and young would be expected to appear above ground in May. The 2002 breeding season, including testes development for several months prior to February, and through the summer following is referred to as Year 1. Late 2002 through 2003 is referred to as Year 2. Thus in Year 1, squirrels were immunized beginning almost 6 months prior to and through the autumn following the breeding season. In Year 2, squirrels were immunized at least 5 months prior to the breeding season and some had been immunized for about 16 months.

### Vaccine Preparation and Immunization Procedure

The vaccine consisted of a synthesized GnRH peptide, EHWSYGLRPG with a glycine and a cysteine added at the carboxyl end, coupled to keyhole limpet hemocyanin (KLH) by the cysteine and emulsified with AdjuVac™, an adjuvant designed at the National Wildlife Research Center. The vaccine was prepared as previously described by Miller et al. (2003). Squirrels were placed in a handling bag and immunized subcutaneously in the lower back with 0.5 ml of vaccine (200 µg of KLH-GnRH conjugate). Placebo vaccine was prepared the same as the GnRH vaccine with all components except the KLH-GnRH.

### Trapping and Reproductive Status Evaluation

Trapping was accomplished by baiting single door National traps with peanut butter, peanuts, and oats. Animals in traps were moved to a central location for visually examined for reproductive status. Males were examined for testes development based on scrotal development. Females were examined for lactation by teat development as a measure of females giving birth. Each squirrel was uniquely identified with ear tags and released where it was trapped.

### Visual Count Index

The visual count index was determined by counting squirrels with binoculars at a distance to limit disturbance to the animals. Counts were done multiple times and at different times of day. The index consisted of the maximal counts obtained during the month. Juveniles and adults were recorded separately and the index reported as a ratio of juveniles per adult.

## RESULTS

### Trapping and Immunizations

In 12 months, 272 squirrels were trapped at the treatment site. Of those squirrels, 127 were trapped at least twice, for a total of 608 trapping events. A total of 229 squirrels were immunized with the GnRH vaccine. As more squirrels were immunized, it became increasingly difficult to trap naive individuals (Figure 1). Some of the naive animals were not immunized because either there was insufficient vaccine available in the field, or they escaped before being immunized. The first immunizations were administered in late August 2001. Trapping continued through the course of this study and immunization continued through September 2002. Less time was spent trapping at the control site, but 57 animals were trapped and 36 animals immunized with placebo vaccine.

The amount of effort required to trap and handle squirrels varied considerably depending on a variety of factors. Personnel hours required per squirrel immunized was at best around ½ hour and sometimes >5 hours.

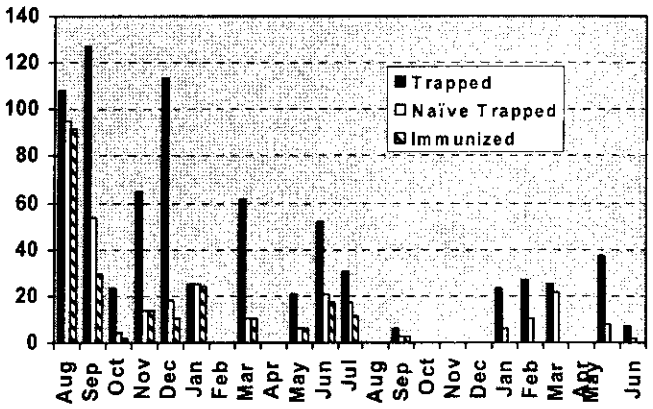


**Figure 1. Trapping of California ground squirrels at the treatment site. Black bars represent the total number of animals trapped, white bars represent trapped animals that had not been previously immunized, and hatched bars represent the number of animals newly immunized in that month. Data is reported for August 2001 to June 2003. Re-trapped animals account for the difference between total trapped and naïve, and naïve animals that were not immunized due to escapes or lack of vaccine account for the difference between the naïve and immunized values.**

### Evaluation of Lactation Status

We trapped squirrels from March through July to determine whether females were lactating. Table 1 shows that females at the control site and unimmunized females at the treatment site had about the same proportion of lactation in Year 1. Squirrels were not trapped during this time period at the control site in Year 2. Squirrels were considered naïve if they were not immunized prior to February of the year they were examined. Squirrels that had been immunized had a much lower proportion of individuals lactating with a reduction of 91% in the first year and 96% in the second year.

### Evaluation of Male Testes Development

Table 2 shows a summary of scrotal testes development for males trapped December through March of each year. In November and December of the first year, the proportion of males with scrotal testes was not substantially decreased in animals immunized 3 or 4 months previously. In March, 3 of 3 unimmunized males, 1 of 1 immunized 3 months previously, 3 of 5 immunized 6 months previously, and 2 of 8 immunized more than 6 months prior had scrotal testes. Overall, immunized males had a 35% less individuals with developed testes in Year 1. In Year 2, an 89% reduction was seen.

### Visual Counting Index

Table 3 shows the results of counting California ground squirrels at both the control and treatment sites. Counts were done in July. Because the sites do not have identical populations, the index is expressed as a ratio of juveniles to adults. In Year 1, the ratio at the treated site was 6% lower than the control site. In Year 2, the ratio was 66% lower at the treated site than the control site.

**Table 3. Visual sighting index of California ground squirrels. An index was obtained by counting California ground squirrels in the month of July.**

| | Juveniles / Adults | |
|---|---|---|
| | Year 1 | Year 2 |
| Control Site | 1.57 | 0.56 |
| Treatment Site | 1.44 | 0.19 |
| % Reduction | 9 | 66 |

## DISCUSSION

### Effects on Fertility

Although GnRH immunization does not affect lactation directly, lactation was a clear measurement for the effect of GnRH immunization on individual ground squirrels; logically, lactation should be a good measure of females that successfully delivered young. The decrease in lactation among treated females indicates that GnRH immunization was effective in reducing fertility. Although only a few females were trapped at the control site for evaluation of lactation, the proportion was similar to the untreated individuals examined at the treatment site, indicating a consistent level of reproduction at the two sites. The difference in the proportion of naïve animals at the treated site that were lactating was very different in Years 1 and 2. But the proportion of immunized animals lactating was similar both years. These results suggest that the vaccine is over 90% effective in inhibiting a female's ability to give birth.

**Table 1. California ground squirrels lactating during March through July. Squirrels trapped at the treatment site are separated into individuals that were immunized prior to February of the year they were evaluated ("Immunized") and those unimmunized as of that time ("Naïve").**

| Year | Site | Immunization Status | Lactation (Number of Females) | | |
|---|---|---|---|---|---|
| | | | Yes | No | % |
| 1 | Control | Control | 2 | 5 | 29 |
| | Treatment | Naïve | 13 | 26 | 33 |
| | | Immunized | 1 | 33 | 3 |
| | | | | Reduction: | 91 |
| 2 | Treatment | Naïve | 5 | 0 | 100 |
| | | Immunized | 1 | 24 | 4 |
| | | | | Reduction: | 96 |

**Table 2. Testes development in California ground squirrels trapped December through March. Squirrels were separated into groups of individuals immunized before the November prior to evaluation ("Immunized") and those that were unimmunized later than that time ("Naïve").**

| Year | Site | Immunization Status | Testis Development (Number of Males) | | |
|---|---|---|---|---|---|
| | | | Scrotal | Abdominal | % |
| 1 | Treatment | Naïve | 13 | 8 | 62 |
| | | Immunized | 8 | 12 | 40 |
| | | | | Reduction: | 35 |
| 2 | Control | Control | 5 | 2 | 71 |
| | Treatment | Naïve | 4 | 1 | 80 |
| | | Immunized | 1 | 10 | 9 |
| | | | | Reduction: | 89 |

**Timing of Immunization**

Since sperm take about 60 days to develop (Fagerstone and Matschke 1977), the testes must develop well before the beginning of the breeding season. This is apparent by the number of males with scrotal testes in November and December. The effectiveness of GnRH immunization in males in the first year may appear deceptively low when using scrotal testes development as a measure. The cut-off we used for dividing the squirrels into immunized and unimmunized squirrels is arbitrary and may be appreciably less than the time required for a sufficient immune response for contraceptive effect. In fact, the earliest immunizations were done at the end of August and there may not have been sufficient antibody titers by November and December to prevent testes development in a majority of the males. As the titer increases enough to block GnRH, testosterone would no longer be produced and sperm development would be halted even though testes might be visibly scrotal. Atrophy of the testes after testosterone production is inhibited may be a delayed process, since males were observed with scrotal testes several months after the breeding season. Thus, we could have males with scrotal testes that were actually infertile due to the immunization by the time breeding season arrived. No immunizations were given within several months of evaluation in Year 2. In this case, the immunizations had time to take effect, and the level of effect was about the same as seen in female lactation levels.

It appears that a single immunization takes several months to be effective. Using 3 months as a minimum time for the majority of immunizations to take effect, females would need to be immunized by the middle of November for the effect to be seen in the first breeding season and males possibly earlier than that. Because the effect of immunization on lactation in individual squirrels occurred at the same levels in Year 1 as in Year 2, we can conclude that the immunization effect lasts at least a year and a half. If immunity did not last that long, the squirrels immunized at the initiation of the study would have become fertile and the reduction in proportion of immunized animals lactating would have increased.

**Population Effects**

Based on our visual count index, there was little effect on population increase the first year. This is probably due to the lower level of squirrels that were effectively immunized at that point in time. A 66% reduction in young born the second year is still not as good as we would like to see. The limiting factor seems to be the number of animals that can be trapped and immunized.

We did not do population determinations as part of this study, so effects on the overall population over time are theoretical. Control of population levels will entail several major factors: the proportion of treated individuals that are effectively contracepted, the proportion of individuals that can be treated, and the survival of existing animals (including possible increased survival in treated populations due to decreased competition). In this study, female California ground squirrels immunized against GnRH were nearly all contracepted. The practical level of the population that can be treated is more difficult to predict and a balance between effort and effect will have to be reached. For the sake of discussion, if we assume that treating 80% of the population is reasonable and further guess that the decrease in fecundity will result in as much as double the survival of offspring due to decreased competition, we would estimate that the remaining 20% of fertile individuals would have 40% of the number of offspring an untreated population would have, or a 60% reduction. These assumptions seem quite conservative and actual results could easily be better. How quickly a reduction in offspring would result in a population decrease if maintained over time will be dependent on the mortality rate, but should be obvious within a squirrel's lifespan (Knipling and McGuire 1972).

**Usefulness for Field Application**

This study indicates that contraception may be a useful tool in population control. In situations such as the park in this study where relocation is not possible and poisoning is banned, this may be a feasible option. For the effect of immunocontraception to be maximized, the time required for sufficient immunity to develop and the duration of the response will need to be determined for each species. The practicality of injectable immunocontraception will be primarily determined by the feasibility of treating a sufficient proportion of the population. Contained areas that are accessible and with minimal immigration will probably be the most amenable locations for this type of management. The cost of the vaccine itself will most likely be much less than the cost of trapping and handling the squirrels, at least for paid workers.

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