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Abstract: Immunocontraception technology appears to have viable application in wildlife damage management. However, administration of these vaccines is presently performed by syringe injection or remote delivery by darts or bio-bullets. In order for immunocontraception to be successful for broad scale application to free-roaming animals, the vaccine must be delivered in an oral form. Recent advances in molecular biology, immunology, and pathology of mucosal infection gives us tools to develop effective oral vaccines. Oral immunocontraceptive vaccine encapsulated in adhesive liposomes or non-virulent live vectors holds promise as a practical approach for immunocontraception of free-roaming wildlife. Issues of safety, species specificity, regulatory constraints, and field application of the vaccine will need to be addressed.

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Key words: immunocontraception, oral vaccine, over-population, population control, wildlife damage management.

Over-population is a worldwide problem in humans, mammals, and birds. In the United States (US) it is often associated with controversy; in humans it often concerns the issue of abortion. In the animal arena, the issue may be associated with killing of animals. Residues from pesticides or steroids entering the food chain are also controversial. Still, the need for population control continues. New insights into hormonal control of reproduction and advances in vaccine technology have brought to the forefront the development of birth control vaccines, technically known as immunocontraceptives. Considerable research has demonstrated that fertility can be controlled by producing antibodies to key reproductive hormones, thus inactivating them (Talwar 1987). The ideal control is to develop a vaccine that would prevent conception, while not affecting other reproductive functions (such as ovulation and secondary sex characteristics.)

Overpopulation of white-tailed deer (*Odocoileus virginianus*) is an increasing problem in some sections of the US. Deer populations continue to increase in many northeastern states despite an increasing number of hunters and more liberal harvest regulations. A 1990 national survey of Animal Damage Control (ADC) program directors showed that ungulates were a significant problem in 35 of the 50 states resulting in damage to forest and agricultural crops and safety hazards related to motor vehicle incidents (Packam and Connolly 1992). In 1994, the US white-tailed deer population was estimated to be 15 million and growing, the highest level this century (Curtis and Richmond 1994). It is important to keep the deer population in balance with its natural habitat.

Traditionally, sport hunting has been used to manage white-tailed deer populations. Encroachment of man into rural areas (e.g., building new homes on 0.8-1.2 ha [2 to 3 ac]) cleared areas has increased the amount of land closed to hunting. Two-thirds of available hunting lands exist in private ownership. This prompts the need to seek alternative methods of population control.

Several alternatives to public hunting have been used. They include translocation, fencing, repellents, lure crops, and crop compensation. These methods have either been ineffective, impractical, or too expensive to be considered as viable alternatives to hunting programs.

IMMUNOCONTRACEPTION

Contraception has been studied as a viable bioalternative to current deer population management methods through the use of synthetic steroids (Roughton 1979). Several attempts to control deer reproduction with synthetic estrogens and progestin have either been ineffective or impractical for field use. Synthetic estrogens and progestins are effective as contraceptives, however, the need to feed the steroids daily make them impractical in field situations. Steroids are also carried through the food chain which makes them ecologically undesirable.

Recent advances in molecular biology and immunology have provided us new tools such as immunocontraception as a means of contraception. Immunocontraception vaccines control fertility by stimulating the production of antibodies against gamete proteins or reproductive hormones. These antibodies interfere with the normal biological activity of these reproductive proteins or hormones (Talwar 1987).

This approach is a natural process in the sense that antibodies induced in the target animal interfere with reproduction without the need for constant medication with synthetic compounds.

Immunocontraception holds the promise to overcome the drawbacks found in the use of steroids for contraception. The immunological approach to contraception is attractive because it requires only periodic vaccination (Turner and Kirkpatrick 1991).

Reproductive Hormones and Proteins

Gonadotropin releasing hormone (GnRH), produced in the brain by the hypothalamus, controls the release of the pituitary reproductive hormones; follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones in turn control the hormonal function of the gonads (ovaries and testes). Antibodies produced to this hypothalamic hormone will reduce the circulating level of biologically active GnRH, thereby reducing the release of subsequent reproductive hormones. The reduction of these hormones results in atrophy of the gonads, resulting in infertility of both sexes.

The zona pellucida (ZP) is an acellular glycoprotein surrounding the oocyte, located between the oocyte and the granulosa cells on the outer surface. Antibodies to this glycoprotein layer result in infertility by one or both of these actions: 1) blocking sperm binding to the ZP layer, and 2) interference with oocyte maturation. In order for a sperm to fertilize the egg, it must first bind to a receptor on the ZP. An enzyme in the sperm breaks down the ZP and allows the sperm passage into the egg. Antibodies to the ZP prevent fertilization by interfering with the binding of the sperm, or the ZP antibodies can interfere with oocyte/granulosa cell communication and result in the death of the developing oocyte (Dunbar and Schwoebel 1988).

Antibodies can be produced to sperm head proteins which normally bind to the ZP receptor on the oocyte. These antibodies are produced in the female and are available to bind to sperm present in the oviduct. Sperm protein immunocontraception is being researched for contraception of the red fox (*Vulpes vulpes*) and the rabbit (*Sylvilagus* spp.) in Australia (Morell 1993, Tyndal-Biscoe 1991).

Chorionic gonadotropin (CG) hormone, which is produced by the implanting embryo, induces the corpus luteum to continue production of the progesterone required for maintenance of pregnancy. Antibodies to CG reduce blood circulation levels of this hormone and therefore prevent implantation of the fertilized egg. Reproduction can be blocked at many sites in the reproductive process, however, the above examples represent the most researched sites (Griffin 1992, Jones 1983).

The Immune Self

The neonate vertebrate immune system develops a recognition of "self" proteins, carbohydrates, and hormones. This self recognition is essential for survival. Antibodies against "self" is an abnormal destructive process as demonstrated in diseases like multiple sclerosis and arthritis . However, the production of antibodies against pathogenic bacteria and viruses is essential for survival. The respiratory and intestinal mucosal surfaces contain various white blood cells (lymphocytes) responsible for generating specific immune responses. In the small intestine, groups of lymphoid cells known as Peyer's patches (PP) serve as "quality control inspectors" sampling bits of food proteins and microorganisms as they pass through. The entire immune system is in constant surveillance to determine self versus foreign and, as in the digestive tract, particles and organisms are examined and either tolerated or attacked by antibodies.

Anti-fertility vaccines are directed against self reproductive antigens (hormones or proteins) to which the recipient normally is immunologically tolerant. These antigens are made non-self or foreign by coupling them to a protein that is foreign to the animal. As the animal samples the conjugated self-foreign protein, antibodies are produced to its own reproductive proteins and hormones. This induced immune response against self is the key to immunocontraception. Infertility lasts as long as there are sufficient antibodies to interfere with the biological activity of the targeted hormone or reproductive protein. This duration is usually 1 to 2 years.

VACCINE ADMINISTRATION

Traditional delivery of vaccines has been by subcutaneous or intramuscular (IM) injection. In order to achieve this form of delivery in the free roaming animal, the vaccine must be delivered remotely by a dart or a bio-bullet (Garrott et al. 1992, Kirkpatrick et al. 1990, Turner et al. 1992). While this method may be effective in certain confined locations, it proves impractical when dealing with wildlife populations in large open areas.

Oral delivery of vaccines has received little attention for human vaccination because it has required larger quantities of vaccines and has been less predictable than subcutaneous and IM routes. In mammals, oral immunization takes place in the pharyngeal immune follicles including the tonsils, and in the small intestine. There are thousands of immune follicles throughout the small intestine with a higher concentration in the distal portion in most species. Vaccines, being protein in nature, are rapidly digested in the stomach when given orally, therefore, immunization must occur in the pharyngeal area or the vaccine needs a protective capsule to survive the stomach and be released in the small intestine (McGhee et al. 1992).

Enteric-coated capsules are commonly used for delivery of drugs to the small intestine. Enteric capsules are resistant to acid, but are soluble in the alkaline solution of the small intestine. They provide only one-half of the formula of effective antigen delivery in the small intestine, that is protection from the stomach acid, since they generally cannot be made small enough to be taken up by the PP. Also, there is another problem with enteric-coated vaccines. They can get the protein past the stomach, dissolve, and release the antigen in the small intestine, but proteolytic enzymes in the small intestine may digest these proteins into non-immunogenic peptides before they are absorbed by the immune cells. The safest way to deliver the antigen orally is to protect it until it is taken up by the PP and delivered to macrophages. Combining 2 approaches: 1) enteric coating using delivery vehicles that slow the intestinal degradation of the antigen and 2) targeting vaccine design to have enhanced attachment to the immune follicles in the small intestine, could lead to an effective antigen uptake and potentiation of mucosal immune response.

Recent understanding of the mechanisms by which pathogenic virus and bacteria colonize and infect the intestinal tract has given us new tools to develop successful, safe, non-live or attenuated live oral vaccines. For example, a bacteria must survive the stomach's acid and proteolytic enzymes

in order to successfully infect the small intestine. After surviving the stomach, it must have surface adhesive properties allowing it to adhere to and colonize the intestinal wall, resulting in an infection. Bacteria without these adhesive properties will be carried out of the gut with the undigested food material.

Liposomes are spherical, artificial biological membranes made up of phospholipids and cholesterol. The liposome membrane contains lipids, chosen for their stability in the gastrointestinal tract, which protect the antigen placed inside during synthesis from gastrointestinal degradation. Cholesterol in the membrane stabilizes it and makes it attractive to macrophages in the PP where the liposome is avidly taken up because of the membrane's lipophilic nature. Because of the nature of the membrane, the liposome simulates a microbial cell when presented to the immune system. The liposome acts as an antigen microcarrier capable of targeting the antigen directly to the PP.

However, before the liposome can be taken up by the macrophages, it must bind to the mucosal surface of the intestine, otherwise it will be swept out with the undigested food material. This mucosal adhesive property increases the mucosal uptake resulting in greater efficiency and a smaller oral vaccine dose. The most common liposome adhesive is the bacterial lectin cholera toxin (CT), a member of a family of enterotoxins produced by several strains of enteropathogenic bacteria (Holmgren et al. 1992). Lectins have multiple binding sites and can bind to receptors on the liposome as well as intestinal receptors.

Recent advancements in molecular biology and immunology have provided us with new tools such as "live vectors" as delivery vehicles. The most prominent use of this technology in wildlife management is the use of live vaccinia virus to orally deliver rabies vaccine to raccoons (*Procyon lotor*) and foxes. The attenuated vaccinia virus, a member of the pox viruses, was used as a vaccine against small pox for over 20 years. Using recombinant genetic engineering, the gene responsible for the encoding of the glycoprotein rabies virus was inserted into the vaccinia virus by the Wistar Institute. This recombinant pox virus, when given orally, is able to vaccinate the target animal against rabies. The tonsil lymphoid tissue is thought to initiate the immune response in these target animals.

IMMUNOCONTRACEPTIVE STUDIES AT DWRC

In 1991, Denver Wildlife Research Center (DWRC) initiated research to develop immunocontraceptive vaccines to address problems caused by damaging species of wildlife. The research and development focus has been on synthetic vaccines for oral immunization of white-tailed deer, wild rats (*Rattus* spp.), starlings (*Sturnus vulgaris*) and brown-headed cowbirds (*Molothrus ater*). If funds are available, other species will be added to the research.

Initial delivery of vaccines was done by injecting captive animals with various preparations as mentioned in this paper. Rats sterilized with GnRH vaccine remained sterile for over a year. White-tailed deer vaccinated with ZP of porcine origin remained sterile for 1-3 years. By vaccinating with an avian specific GnRH, we have shut down spring production of testosterone in male brown-headed cowbirds.

Our initial work centered on development of the vaccine and measuring immune and hormonal levels in response to systemic vaccination. Our goal is to deliver the vaccine orally. The DWRC has successfully sterilized Norway rats (*Rattus norvegicus*) by oral immunization with GnRH encapsulated in an adhesive liposome which was designed at DWRC.

EVALUATING THE EFFECTIVENESS OF THE CONTRACEPTIVE VACCINE

In addition to breeding trials, effectiveness of immunocontraceptive vaccines is assessed by measuring serum progesterone, testosterone, and antibody titers. Reduction in hormone level as well as elevated antibody levels should correlate with sterility of the animal. All immunocontraception vaccines presently being studied result in some behavioral changes. These behavioral responses vary from total reduction in sexual function in both males and females to multiple estrus in the female immunized with ZP.

ORAL VACCINE DELIVERY SYSTEM

The rapidly expanding potential of recombinant DNA technology allows the use of recombinant bacteria or viral vectors to deliver the vaccine to wildlife. However, it is impractical to inject every animal either under restraint or remotely by a dart gun; therefore, oral vaccination represents the only practical method for mass vaccination of free roaming animals. The oral vaccine and/or bait should be species specific, and must be delivered to the tonsils (pharyngeal tissue) or the Peyer's patches in the small intestine. Oral vaccination is generally less efficient than an injection in relation to the quantity of antigen used. Recombinant live bacteria or viral vectors are probably the only effective way to deliver these antigens. The safety of the oral vectors will need to be proven to the public.

Recent worldwide interest in oral vaccines for cholera toxin and the AIDS virus is rapidly providing technology and dialogue to the above questions that can be applied to the animal vaccine arena.

PUBLIC ISSUES

A number of issues must be addressed before a program is developed to administer these vaccines. Certain animal welfare and animal rights issues must be answered. Is it moral to alter the animal's reproductive system? Is there any unusual pain involved with vaccination? Are we changing the usual behavior of these animals? Can any residue vaccine be carried through the food chain? What are the effects on non-target animals? Will non-target animals, perhaps an endangered species, become sterilized?

What are the human safety issues involved in such a program? Can a human accidently ingest the oral vaccine and become sterilized? How will public education on technology be organized? After a vaccine has been produced in production scale, who controls the delivery of the vaccine? Is the use of the vaccine to be controlled at the federal, state, or local government level?