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IMMUNOCONTRACEPTION AS A TOOL FOR CONTROLLING REPRODUCTION IN COYOTES

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Abstract: The development of immunocontraception as a tool for population management of coyotes (*Canis latrans*) and reduction of coyote predation may provide an environmentally safer alternative to pesticides. Because they are proteins, immunocontraceptive vaccines do not persist in the environment or bioaccumulate in the food chain. The National Wildlife Research Center (NWRC) will examine the effects (immunological, hormonal and behavioral) of treating penned coyotes with 2 immunocontraceptive vaccines: porcine zona pellucida (PZP) and gonadotropin releasing hormone (GnRH). Initial studies will be conducted using traditional subcutaneous injections; however, the goal is to develop an orally-deliverable immunocontraceptive vaccine as an alternative tool for coyote population management.

Livestock predation by coyotes is a chronic conern of many sheep and goat ranchers. A 1990 survey estimated that, of the nearly 6 million lambs born in the 16 western states, 549,000 lambs died from all causes (Connolly 1992). Nearly 60% of the losses were a result of predators. Coyotes were the main culprit, accounting for 70% of the predator-caused mortalities. The economic impact on producers and consumers in 1990 was approximately $11.4 million. Despite intensive historical control efforts in livestock production areas, and despite sport hunting and trapping for fur, coyotes continue to thrive and expand their range, occurring widely across North and Central America.

Scientists at the National Wildlife Research Center and its predecessor laboratories have conducted research for over 50 years on the problem of livestock predation by coyotes, and on developing methods to minimize predation losses. Available techniques include husbandry practices, shooting, trapping, frightening devices, livestock guarding dogs and toxicants (Fall 1990). None of these control methods is completely practical or effective in all of the diverse situations in which coyote predation on livestock occurs. Also, as the costs of labor-intensive skills and approaches continue to increase, new techniques are needed. Further, coyotes are viewed increasingly by the public as a desirable wildlife species. Accordingly, effective nonlethal methods are being sought for resolution of predation problems.

Immunocontraception has been suggested as l nonlethal technique with application for reducing coyote numbers in areas where they are causing depredation losses, or for managing the predatory behavior of territorial pairs (Knowlton 1989). However, private industry has had little economic incentive to develop new materials for this use because of the small quantities of materials that would be used in predation control. This situation with immunocontraception vaccines parallels that for toxicants and other coyote predation control products (Linhart et al. 1992).

Basics of immunocontraception

The neonatal vertebrate immune system develops a recognition of "self" proteins, carbohydrates, and hormones. This self recognition is essential, since the production of antibodies against pathogenic bacteria and viruses is necessary for survival. However, the formation of antibodies against "self" can be an abnormal destructive process, e.g., diseases like multiple sclerosis and arthritis.

The entire immune system is in constant surveillance to determine "self" vs "foreign" proteins. For example, in the digestive tract, particles and organisms are examined and either tolerated or attacked by antibodies. The respiratory and intestinal muco-

sal surfaces contain various white blood cells (lymphocytes and macrophages) that are responsible for generating specific immune responses. In the small intestine, groups of lymphoid cells known as Peyer's patches (PP) sample bits of food proteins and microorganisms as they pass through to determine if an immune response will be directed against the incoming organism or food particle.

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 recognized as foreign to the animal. As the animal' immune system examines 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. The infertility lasts as long as there are sufficient antibodies to interfere with the biological activity of the targeted hormone or reproductive protein, usually 1-2 years.

Reproductive hormones and proteins involved in immunocontraception

Immunocontraceptive vaccines can control reproduction at various stages. They can interrupt the reproductive activity of both sexes by (a) interfering with the biological activity of hormones, (b) blocking sperm penetration of an ovulated egg, or (c) preventing implantation and development of a fertilized egg.

Gonadotropin releasing hormone (GnRH) is produced in the brain by the hypothalamus and controls release of the pituitary reproductive hormones follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones in turn control the hormonal functions of the gonads (ovaries and testes). Antibodies to the hypothalamic hormone will reduce the circulating level of biologically-active GnRH, thereby reducing the release of subsequent reproductive hormones. The reduction or absence of these hormones leads to atrophy of the gonads, resulting in infertility in both sexes. Both avian and mammalian forms of GnRH have been identified.

The zona pellucida (ZP) is an acellular glycoprotein surrounding the egg or oocyte. It is located on the outer surface of the egg between the oocyte and the granulosa cells. Antibodies to this glycoprotein layer result in infertility by 1 or both of these actions: (a) blocking sperm from binding to the ZP layer, and (b) interfering with oocyte maturation. 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 ovum. Antibodies to the ZP also prevent fertilization by interfering with oocyte-granulosa cell communication, resulting in the death of the developing oocyte (Dunbar and Schwoebel 1988).

Since protein in the sperms' head normally bind to the ZP receptor on the oocyte, antibodies to these sperm proteins can be produced, by vaccination in the female that are available to bind to sperm in the oviduct. This prevents sperm from binding to the ZP receptor. Sperm protein immunocontraception is being investigated for contraception of the red fox and the rabbit in Australia (Morell 1993, Tyndale-Biscoe 1991). A ZP protein has not been identified in avian species, nor has the cross-reactivity of PZP been tested in avian species.

Chorionic gonadotropin (CG) hormone, which is produced by the implanting embryo in some species, induces the corpus luteum to continue production of progesterone which is required for the maintenance of pregnancy. Antibodies to CG reduce blood levels of this hormone and thereby prevent implantation of the fertilized egg.

The riboflavin requirement of the developing embryo is satisfied by active transport of this water-soluble vitamin across the placenta. This transport is provided by a gestational-specific carrier protein called riboflavin carrier protein (RCP). RCP plays a pivotal role in embryo development in avian and mammalian species. Antibodies formed against RCP interfere with placental transfer of riboflavin, thereby preventing development of the early embryo. This technology probably would result in the least change in social behavior of the target species of any of the proposed vaccines (Natraj et al. 1987, 1988).

Reproduction can be blocked at many sites in the reproductive process; the above examples are the sites where most investigative work has been done. Behavioral and social changes in target animals resulting from specific vaccines may dictate the

vaccine of choice in each situation (Jones 1982, Griffin 1992).

Methods of administering vaccines

Subcutaneous or intramuscular (I.M.) injection are the traditional forms of vaccine delivery. In order to accomplish I.M. injections in free-roaming animals, the vaccine must be delivered by a dart or a "bio-bullet" (Kirkpatrick et al. 1990, Turner and Kirkpatrick 1991, Garrot et al. 1992, Turner et al. 1991, 1992). While these methods may be effective in certain confined locations, they are impractical when dealing with mobile wildlife populations in large open areas.

Except for the oral polio vaccine introduced by Dr. Sabin in the 1950s, oral vaccination has received little attention for humans because it requires larger quantities of vaccine and is less predictable than subcutaneous or I.M. routes. In mammals, oral immunization takes place in the pharyngeal immune follicles (e.g., 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 digested rapidly in the stomach when given orally; hence, immunization must occur either in the pharyngeal area or the vaccine needs a protective capsule to survive passage through the stomach then be released in the small intestine (McGhee et al. 1992).

The safest way to deliver the antigen orally is to protect it until it is taken up by the PP and delivered to macrophages. A combination of 2 approaches could lead to effective antigen uptake and potentiation of mucosal immune response: (a) enteric coating of the antigen resulting in delivery vehicles that prevent degradation in the stomach but allow absorption in the intestine, and (b) designing the vaccine to have enhanced attraction to the immune follicles in the small intestine.

Recent understanding of the mechanisms by which pathogenic viruses and bacteria colonize and infect the intestinal tract has provided new insights for developing successful and safe attenuated live or killed, oral vaccines. For example, a bacteria must survive the stomach's acid and proteolytic enzymes to successfully infect the small intestine. After surviving intact through the stomach, it must have adhesive properties which allow it to adhere to and colonize the intestinal wall, resulting in an infection. Bacteria without adhesive properties will be carried out of the gut with the waste material.

Liposomes are spherical, artificial biological membranes made up of phospholipids and cholesterol that can be used to protect oral vaccines from digestive tract degradation. Since the liposome membrane contains lipids, which are stable in the gastrointestinal tract, an antigen placed inside during liposome synthesis is protected from gastrointestinal degradation. Cholesterol in the membrane adds stability and makes it attractive to macrophages in the PP where the liposome is taken up rapidly because of the membrane's lipophilic nature. This characteristic of the membrane causes the liposome to simulate 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 a 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 waste material. This mucosal adhesive property increases the mucosal uptake efficiency, thus requiring a smaller oral vaccine dose. The most commonly used liposome adhesive is a nontoxic form of 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 to 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 the live vaccinia virus to deliver rabies vaccine orally to raccoons *(Procyon lotor)* and foxes (*Vulpes vulpes*). The attenuated vaccinia virus, a member of the pox viruses, was used as a vaccine against smallpox in humans for over 20 years. Using recombinant genetic engineering, the gene responsible for encoding of the rabies virus glycoprotein was inserted into the vaccinia virus by scientists at the Wistar Institute. This recombinant pox virus, when given orally, was able to vaccinate the target animal against rabies. The tonsil lymphoid

tissue is thought to initiate the immune response in these target animals (USDA-APHIS 1991).

Live viral vectors potentially can be used to deliver a contraceptive vaccine. This delivery system is currently being tested in Australia (Tyndale-Biscoe 1991).

Potential of immunocontraception in coyote management

Immunocontraception as a technology is available today, but only for use in a laboratory setting and pen studies. Immunocontraceptive vaccines are being produced in limited quantities and animals injected with these vaccines become infertile for 1-3 years.

The development of a practical, cost-effective immunocontraceptive vaccine for coyotes is a multi-year, multi-task project. The first task the NWRC will undertake will be to determine the immune, hormonal and behavioral responses to non-species-specific PZP and GnRH immunocontraceptive vaccines. Using serum from known immuno-sterilized and fertile coyotes from the above study, a new mimotope assay will be used to determine portions of the PZP active in sterilizing the coyote. This new test may hold promise for finding a PZP peptide specific to coyotes. These species-specific peptides could then be used to develop a species-specific ZP vaccine. GnRH will continue to be studied where species specificity is not critical.

Some important behavioral questions related to the effects of contraception on pair formation, pair bond maintenance, breeding behavior and territorial defense need to be addressed. The answers may dictate in part the choice of vaccines to be developed for immunocontraception in coyotes.

Practical use of immunocontraception for controlling free-ranging coyote populations will have to involve oral delivery of the vaccine. The technology for developing oral vaccines is in its infancy. However, because of a worldwide need for oral vaccines against cholera and the HIV virus, rapid progress is being made in this area. Oral immunization using liposome or bacterial vectors will be the goal of the NWRC. Vaccines encapsulated in liposomes will provide protection from the gastrointestinal environment and can induce a 500-fold greater oral immune response as compared to free antigens. We plan to develop liposomes with a cholera-toxin-B subunit on their surface to mimic the adhesive properties of intestinal pathogens and ensure optimal host immune response.

Finally, prior to field use, U. S. Food and Drug Administration approval of the safety and efficacy of this new vaccine will be needed. Extensive laboratory, field and product testing will be required before this or other materials are available for use in management programs.

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