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The raging North American controversy over the reintroduction of wolves into the ecosystem of the greater Yellowstone National Park area exemplifies the emotive relationship between humankind and the Carnivora. What forces act in concert to portray this much maligned Order in unfavorable light? Control of free-ranging carnivore populations by *Homo sapiens* has been practiced for centuries as part of a pastoral lifestyle, with the intent of protecting one's own life and livelihood from becoming freshly killed prey in the onslaught from mammalian competitors. Traditionally, control is equated most commonly with population reduction through direct elimination of individuals (e.g., typically social canids or solitary large-bodied felids) via lethal means including shooting, poisoning, trapping, gassing of dens, and habitat modification (Lewis 1968, U.S. Department of Agriculture 1992). In addition to reducing direct predation upon domestic livestock (sheep and cattle losses alone in the United States are estimated in excess of $80,000,000 annually [U.S. Department of Agriculture, National Agricultural Statistics Service 1991]), other perceived beneficial aspects of free-ranging carnivore population reduction include conservation of endangered species, such as Australian marsupials, subject to predation by introduced European red foxes (Boyle 1994), conservation of otherwise "desirable" species (game fowl and wild ungulates), and the alleviation of objectionable human–carnivore interactions (Wynne–Edwards 1964). Today, such interactions range from local citizen complaints of seemingly frivolous or nuisance wildlife encounter—raccoon disruption of a backyard songbird feeder, bear vandalism at vacation homes, etc.—to significant global public health issues (such as animal bite from the stray dog) and related human mortality either directly from overt injury or indirectly from exposure to a plethora of zoonoses, such as rabies or echinococcosis (Beran 1994). Nevertheless, a "manageable" number of mammalian carnivores is clearly viewed as beneficial when they serve human desire for sport, pelts, companionship, etc. Moreover, sound ecological, economic, and ethical arguments weigh against sole reliance upon lethal mechanisms to resolve such conflicts. A comprehensive approach to conflict management, rather than a narrow focus only upon overt, uncompromising predator decimation, is a valid and potentially more sustainable strategy to manage human–wildlife conflicts. Can targeting and controlling carnivore proliferation resolve the dilemma and validate this premise of alternative, nonpernicious intervention?

Historically, control of mammalian reproduction has been primarily directed toward domestic companion animals and livestock. Contraception has typically consisted either of surgical neutering of individuals, hormonal manipulation of reproductive function, or simple physical separation of the sexes. While the neutering of feral cats has been suggested as an alternative to elimination (Zaunbrecher and Smith 1993), these techniques, which are suited for management of individual reproductive function, may only rarely be applicable to most free-ranging carnivore populations, given the constraints of diverse species distribution and abundance. In contrast, oral delivery of a contraceptive agent for reproductive control among wild carnivores may be more feasible; initial efforts were reported as early as three decades ago (Balser 1964).

The observation of naturally occurring antisperm antibodies in a small proportion of humans has generated interest in the recruitment of the immune system for reproductive modulation (Aitken et al. 1993). Some postulated advantages of immunologically mediated contraception may be (1) economical vaccine production by recombinant techniques, (2) ease of administration, (3) relatively few side effects, and (4) a higher degree of biological specificity than traditional chemical drug delivery, which may have a broader phylogenetic and physiological spectrum of activity. From an ecological perspective, one potential advantage of wildlife immunocontraception would be to minimize deleterious effects of free-ranging carnivores by reducing or stabilizing total numbers, while avoiding vacant niches inherent to lethal reduction. A nonreproductive adult would inhibit ingress of new, fully reproductive individuals from surrounding areas (Porter et al. 1991). Arguably, one

weakness of the immunocontraception prospectus is individual variation in the immune response, which may lead to unpredictability regarding the duration and magnitude of effect in a particular animal. However, if a measurable effect among a local population is achieved, some variation among individuals may be acceptable.

Typically, oocyte and sperm antigens are sufficiently compartmentalized so that an immune response is not normally elicited; however, these antigens are clearly immunogenic (Haimovici et al. 1992, Liu et al. 1990). In this regard, considerable research has focused on zona pellucida (ZP) antigens (Kirkpatrick et al. 1991 and 1992, Hasegawa et al. 1992, Jones et al. 1992). Despite a highly species-specific interaction between the sperm surface and a glycoprotein component of the ZP, antibodies to the ova of one species inhibit in vitro and in vivo fertilization of another species (Aitken et al. 1993). An unexpected finding from ZP immunization has been the delayed cessation of ovarian cycling from destruction of primordial follicles or essentially induced premature menopause in animal models (Hasegawa et al. 1992, Jones et al. 1992), another potential drawback in the implementation for wildlife.

Alternative approaches have focused upon inducing antibodies to the cumulus oophorus of the conceptus (Tesarik et al. 1990) or disrupting regulatory hormones such as human chorionic gonadotrophin, gonadotropin-releasing hormone, luteinizing hormone-releasing hormone, and follicle-stimulating hormone (Aitken et al. 1993). Additionally, while still the subject is still in the early stages of investigation, some promising results have also been obtained with disruption of spermatogenesis (Grubb 1991). Some of these methods raise complex medical or ethical issues, for human reproductive manipulation because the end result may be essentially abortifacient or complications related to immune-complex formation. Whether these matters would be equally as controversial when applied to a "nuisance" carnivore species has yet to be determined. However, it should be clear that absolute restriction to the species of interest would be optimal.

Other suggested interventions would target levels of reproductive hormone (testosterone or progesterone) directly (Linhart 1964, Linhart et al. 1968, Awoniyi et al. 1992, Moudgal et al. 1992, Talwar et al. 1992, Vanage et al. 1992, Deshmukh et al. 1993, Dowsett et al. 1993, Ladd 1993). The significant limitation of this approach in a free-ranging carnivore population is the potential for an undesirable effect upon sexual behavior, social interactions, and hierarchy (Awoniyi et al. 1992, Moudgal et al. 1992, Dowsett et al. 1993).

To date, no species-specific reproductive antigens have been identified, although unique contraceptive antigens for humans (Aitken et al. 1993), wolves (U.S. Department of Agriculture 1992), red foxes, rabbits, kangaroos (Morell 1993), deer (Porter et al. 1991), wild swine (Fletcher et al. 1990), and many others (Wynne–Edwards 1964), would be of great utility. The apparent conservation of many reproductive antigens among mammalian groups raises the undesirable, even detrimental, potential to unintentionally affect nontarget species, possibly including humans, valuable domestic animals, endangered or threatened wildlife, and nonnuisance carnivore species. In lieu of species-specific antigens, a species-specific vector (plasmid DNA, viral, bacterial, etc.) would be a potential strategy to limit the contraceptive effect solely to the target species. Unfortunately, such carnivore species-specific vectors have also yet to be identified.

The physical delivery of a desired contraceptive may consist of a variety of singly applied or combined approaches. For example, live-trapping of free-ranging carnivores and direct inoculation of a contraceptive may be of some value, particularly in areas where high human–carnivore interaction is problematic and necessitates a response, but complete elimination of carnivores is not desired by human residents, and lethal control is unacceptable. Except for under these limited conditions, the labor-intensive nature of this approach and the poor capture rates of some carnivore species may render this method largely impractical.

Conversely, injection of contraceptive agents may be achieved remotely via a blow gun, dart gun, or similar device. This is currently a procedure in progress for an insular population of feral horses off the eastern mid-

Table 1. Oral vaccination of carnivores with recombinant viruses

Agent	Species (common name)	Reference
Vaccinia-Rabies Glycoprotein Recombinant Virus		
Family Canidae	*Vulpes vulpes* (red fox)	Blancou et al. (1986)
	Canis lupus (domestic dog)	Blancou et al. (1989)
	C. latrans (coyote)	Artois et al. (1990)
	Alopex lagopus (arctic fox)	Chappuis and Kovalev (1991)
	Nyctereutes procyonoides (raccoon dog)	Chappuis and Kovalev (1991)
	Urocyon cinereoargenteus (grey fox)	Rupprecht et al. (1992a)
Family Felidae	*Felis domesticus* (domestic cat)	Blancou et al. (1989)
	Lynx rufus (bobcat)	Rupprecht et al. (1992a)
Family Mustelidae	*Mephitis mephitis* (striped skunk)	Tolson et al. (1987)
	Mustela putorius (ferret)	Brochier et al. (1988)
	Meles meles (European badger)	Brochier et al. (1989)
	Lutra canadensis (river otter)	Rupprecht et al. (1992a)
	Mustela vision (mink)	Rupprecht et al. (1992a)
Family Procyonidae	*Procyon lotor* (raccoon)	Wiktor et al. (1985)
Family Ursidae	*Ursus americanus* (black bear)	Rupprecht et al. (1992a)
Raccoonpox-Rabies Glycoprotein Recombinant Virus		
Family Procyonidae	*P. lotor*	Esposito et al. (1988)
Family Canidae	*C. lupus*	Esposito et al. (1992)
	U. cinereoargenteus	Esposito et al. (1992)
Family Felidae	*F. domesticus*	Esposito et al. (1992)
	L. rufus	Esposito et al. (1992)
Family Mustelidae	*M. mephitis*	Fekadu et al. (1991)
Human Adeno(5)-Rabies Glycoprotein Recombinant Virus		
Family Procyonidae	*P. lotor*	Charlton et al. (1992)
Family Canidae	*V. vulpes*	Charlton et al. (1992)
	C. lupus	Campbell (1994)
Family Mustelidae	*M. mephitis*	Charlton et al. (1992)
Baculo-Rabies Glycoprotein Recombinant Virus		
Family Procyonidae	*P. lotor*	Fu et al. (1993)

Atlantic shore (Kirkpatrick et al. 1991 and 1992). As above, this approach is also largely limited by species secretiveness, tolerance for humans, the accuracy of the operator, and the ability to identify previously inoculated individuals.

Given these limitations, additional methodologies may have to be considered for long-term, widespread carnivore reproductive control. For example, the effectiveness and relative ease of using baits to deliver a biological, rather than lethal chemicals as practiced historically, to wild carnivores has been demonstrated, principally through the wildlife rabies vaccination of several reservoir species in Europe and North America (Johnston et al. 1988, Bachmann et al. 1990, Brochier et al. 1990, Rupprecht et al. 1992a, Winkler and Bogel 1992, Campbell 1994). This example of wildlife rabies vaccination has often been cited over the last decade to document the degree of sophistication achieved in reaching free-ranging carnivore populations. To date, these field systems involve either modified live rabies viruses or recombinant orthopoxvirus vectors that undergo limited replication without perpetuation or apparent adverse effect (at least in the latter viral scenerio) in the targeted host. The advantages of a self-replicating entity are economy and the more reliable induction of an immune response without the need for

multiple doses or adjuvants. Moreover, vectors with wide carnivore host susceptibility (table 1) are advantageous with a disease such as rabies, in which the pathogen is not restricted to a single narrow host niche. For example, a single biologic may be useful for control of rabies in raccoons, red foxes, and coyotes in various geographic areas where rabies strains are perpetuated by different carnivore species. Yet this same precept of broad application may be counterproductive without species-specific expression products, when the effect is immunocontraception in co-occurring species, rather than simply rabies vaccination.

In addition to live virus vaccination, successful oral immunization of raccoons in captivity has also been demonstrated with a baculo–virus system, in which rabies glycoprotein expression in an insect cell culture resulted in sufficient quantities of antigen to immunize animals directly by mouth (Fu et al. 1993). Similarly, raccoons and other carnivores may be orally immunized with inactivated viral preparations (Rupprecht et al. 1992b). While the amount of antigen required may be economically prohibitive given current production limitations, the concept offers a choice avenue of investigation that departs from the traditionalist approach toward a replicative vector, if a restricted reproductive antigen were available.

A self-replicating biologic has an inherent potential for adverse effects that is influenced by host variables, such as species and individual age, immune status, concurrent infectious or metabolic conditions, etc. The latent risk for adverse effects may be nearly immeasurable under traditional laboratory or field conditions. These concerns are particularly relevant to an immunocontraceptive, self-replicating biologic destined for free-choice broadcasting and consumption. While the occurrence of immunocompromised hosts at risk for vaccine exposure may be remote, any self-replicating vector, even a highly attenuated virus, presents increased risks in such a host (Fenner et al. 1988, Hierholzer 1992).

The immunocompromised host scenerio has led to the development of functional animal models. Bosma and Carroll (1991) have identified a single gene mutation in mice that results in the inability to form functional B and T cells in homozygotes. Lacking the capacity for a specific immune response to pathogens and commensal organisms alike, severe combined immunodeficient (SCID) mice must be housed under aseptic conditions in a pathogen-free environment. An inheritable, functionally similar condition occurs in humans. Thus, the SCID model may be particularly useful in the elucidation of events during recombinant viral infection and may contribute toward the knowledge of the overall biosafety of these new biologics (Hanlon et al. 1997). Additionally, such studies may identify critical components of a prophylactic regimen, should adverse effects occur in an immunocompromised host. As a more sophisticated working knowledge of viral genetics is gained, genomic sequences crucial for replication in a particular host may be targeted and eliminated (Tartaglia et al. 1992a and b), increasing species specificity, as well as overall biological safety.

The synergism provided by vaccine vector, bait type, and distribution parameters (density, method, spatiotemporal factors) should ultimately maximize target species contact and minimize nontarget species uptake of a given biological. However, it is difficult to imagine total vaccine restriction to a single carnivore population even under ideal circumstances. Many bait studies have previously demonstrated an effect on species other than the target and implications for nontarget groups, such as domestic animals, humans and nonmammals, despite the original application and intention (Ballantyne and O'Donoghue 1954, Linhart 1964, Lewis 1968, Westergaard 1982, Bachmann et al. 1990, Fletcher et al. 1990, Trewhella et al. 1991). For example, a decade of applied research toward development of a prototype delivery device for oral raccoon rabies vaccination (Rupprecht et al. 1987) in the Eastern United States, resulted in a fishmeal–polymer bait that was readily consumed by a majority of raccoons under laboratory and field conditions (Rupprecht et al. 1992a). Yet variations in bait density (10–100/ha), distribution season, habitat type (barrier island to forested uplands), or method (hand delivery *v.* aerial), targeting ecotones suggestive of high raccoon activity, were unable to exclude consumption by other mammals (Hanlon et al. 1989 and 1993, Hable et al. 1992, Rupprecht et al. 1992a). Viewed as

Table 2. Biomarker[1] detection in nontarget species from fishmeal–polymer bait consumption: Virginia, Pennsylvania, and New Jersey (1990–93)

Species	No. positive/total	Percent
Opossum *(Didelphis virginianus)*	64/95	67
Striped skunk *(Mephitis mephitis)*	13/32	41
Domestic cat *(Felis domesticus)*	6/20	30
Red fox *(Vulpes vulpes)*	2/6	33
River otter *(Lutra canadensis)*	1/5	20
Porcupine *(Erythizon dorsatum)*	3/36	8
Black bear *(Ursus americanus)*	2/198	1
Norway rat *(Rattus norvegicus)*	1/8	13
House mouse *(Mus musculus)*	2/15	13
Rice rat *(Oryzomys palustris)*	2/7	29

[1] Tetracycline analysis from mandibular bone as described by Hanlon et al. (1989).

a composite (table 2), utilization in excess of 100,000 V–RG vaccine-laden baits specifically for raccoons nevertheless demonstrated contact by a variety of other carnivores and a few rodent species (albeit extremely small numbers). Overall biomarker data indicated bait consumption by a limited variety and number of nontarget species with no evidence of consumption by certain others (such as white-tailed deer during hunting season). These results could not have been predicted a priori, without placebo baiting and nontarget species surveillance. Observed nontarget species outcomes from vaccine exposure in the field have ranged from no apparent effect to immunization. However, what does the bait contact rate of a nontarget, competitor species (e.g., opossums) imply, especially if it approximates or exceeds that of the target species [raccoons]? From a disease control perspective, in which no untoward effects have been demonstrated in the nontarget species at issue, the answer may range from simple nuisance to a resultant economic infeasibility, depending upon the degree of interference and the number of vaccine-laden baits not available to the intended species. Clearly, what looks like a trifling matter—say, an overabundance of opossums vaccinated against a given infectious disease—may not be trivial in regard to immunocontraception, in light of species-specific vectors, antigens, baits, etc. This nontarget species contact problem may figure prominently if the species in question is a keystone species.

Long-term results of applying free-choice oral immunocontraception to free-ranging carnivores (and the associated nontarget milieu) are impossible to predict at present with any reasonable degree of certainty, as regards either safety or efficacy. For example, the efficacy of oral rabies vaccination among a target population may be assessed by (1) confinement studies with baits followed by challenges, (2) capture of free-ranging animals from a vaccinated area for subsequent laboratory challenge (Rupprecht et al. 1993), (3) measurement of seroconversion among free-ranging animals in an area, or simply (4) surveillance for naturally occurring disease. However, the minimal acceptable levels of these assessment techniques that would predict successful disease control or elimination are not known a priori, nor from present data, nor for a variety of complex ecological settings.

A proportion of the population may not consume baits due to a variety of factors. Some heritable behavioral traits, such as temerity in consumption or total avoidance of novel items, like artificial baits, may play a role in the inability to reach a segment of the targeted population. It follows that a particular cohort with a behavioral trait of bait avoidance may gain a competitive advantage. The result would be increasing difficulty in reaching this remaining, actively prepetuating segment of the population via baits. This scenario may be particularly troublesome given the high reproductive capacity of some species. Additionally, because no vaccine is completely efficacious in all individuals, it may be possible to selectively favor nonresponders (the perceived "mangey" or "wormy" individuals), due to major histocompatibility restriction, inherited immunodeficiencies, or immunocompromising infectious agents (Nossal 1989). If these latter theoretical demes gain even a minor reproductive advantage within a population, they may eventually initiate or exacerbate disease and related conditions. Amplification of this particular component of a carnivore population may severely restrict genetic diversity, as in present day cheetah (i.e., *Acinonyx)* (Cohn

1986), and subsequently compromise the overall health and viability of the community at large.

To be ultimately successful, an immunocontraceptive control program will intuitively require reaching a majority of individuals; how can the ideal mix of gene frequencies be ensured in light of this seeming conundrum? At first consideration, a readily transmissible, "safe" recombinant agent (while a bane to regulatory authorities) might appear to overcome the limitation of inequitable bait uptake and biological response. Exposure to a readily transmissible vector could approach unity, successfully reaching all members of a particular carnivore population. But, as evidenced by the global emergence and entrenchment of canine parvovirus within domestic and wild canid populations (Parrish 1994), this strategy may have significant uncontrollable and potentially detrimental effects. Even if a so-called species-specific antigen were discovered, geographic containment of such a highly contagious agent could not be assured. How would programs aimed, for example, at red foxes in the New World prevent exchange to red foxes in the Old World, involvement of related subspecies, or spillover to kin in the same genus, given the frequency of transoceanic travel and exotic and endemic species translocations (Rupprecht et al. 1996)? Similar questions could be raised for other taxa—canid, mustelid, viverrid, etc.

In conclusion, incipient investigations toward immunocontraceptive population management are quite intriguing. Their development for free-ranging carnivores appears well motivated and potentially desirable, at first glance, for numerous applications, given the limitations of available alternatives to reconcile the human–predator interface problem. Nonetheless, it will be crucial to proceed from the outset in as prudent a manner as possible, given the above-voiced concerns. It will be necessary to address, in comprehensive fashion, the potential for untoward events, and objectively divest real from perceived risks, much akin to the scientific scrutiny directed toward recombinant biologics more than a decade ago. In concordance with the recommendations of the World Health Organization (1993) in their Consultation on Reproductive Control of Carnivores, future directions of immunocontraceptive research should include continued efforts to develop species-specific bait delivery techniques, and species-specific contraceptive effects, either through antigen or vector. Given these goals, future research would logically involve international, multidisciplinary, collaborative efforts, strongly based upon objective, testable hypotheses. Until then, free-choice broadcasting of nonrestrictive contraceptive biologics may be unconscionable due to, as yet, unpredictable, undesirable, and potentially far-reaching repercussions, not only in the target species, but also in critical nontargets that share this increasingly burdened and now readily traversed globe.

References Cited

Aitken, R. J.; Paterson, M.; Koothan, P. T. 1993. Contraceptive vacines. British Medical Bulletin ro: 88–99.

Artois, M.; Charlton, K. M.; Tolson, N. D.; Casey, G. A.; Knowles, M. K.; Campbell, J. B. 1990. Vaccinia recombinant virus expressing the rabies virus glycoprotein: safety and efficacy trials in Canadian wildlife. Canadian Journal of Veterinary Resarch 54: 504–507.

Awoniyi, C. A.; Kim, W. K.; Hurst, B. S.; Schlaff, W. D. 1992. Immunoneutralization of gonadotropin-releasing hormone and subsequent treatment with silastic implants in rats: an approach toward developing a male contraceptive. Fertility and Sterility 58: 403–408.

Bachmann, P.; Bramwell, R. N.; Fraser, S. J.; Gilmore, D. A.; Johnston, D. H.; Lawson, K. F.; MacInnes, C. D.; Matejka, F. O.; Miles, H. E.; Pedde, M. A.; Voight, D. R. 1990. Wild carnivore acceptance of baits for delivery of liquid rabies vaccine. Journal of Wildlife Diseases 26: 486–501.

Ballantyne, E. E.; O'Donoghue, J. G. 1954. Rabies control in Alberta wildlife. Veterinary Medicine 23: 87–91.

Balser, D. S. 1964. Management of predators with antifertility agents. Journal of Wildlife Management 28: 352–358.

Beran, G. W., ed. 1994. Handbook of zoonoses. Boca Raton, FL: CRC Press. 1,168 p.

Blancou, J.; Kieny, M. P.; Lathe, R.; Lecocq, J. P.; Pastoret, P. P.; Soulebot, J. P.; Desmettre, P. 1986. Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. Nature 332: 373–375.

Blancou, J.; Artois, M.; Brochier, B.; Thomas, I.; Pastoret, P. P.; Desmettre, P.; Languet, B.; Kieny, M. P. 1989. Innocuité et éfficacité du virus recombinant vaccine-rage administre par voie orale chez le renard, le chien et le chat. Annales de Recherches Vétérinaires 20: 195–204.

Bosma, M. J.; Carroll, A. M. 1991. The SCID mouse mutant: definition, characterization, and potential uses. Annual Review of Immunology 9: 323–350.

Boyle, D. B. 1994. Disease and fertility control in wildlife and feral animal populations. Reproduction, Fertility, and Development 6: 393–400.

Brochier, B.; Languet, B.; Blancou, J.; Thomas, I.; Kieny, M. P.; Lecocq, J. P.; Desmettre, P.; Pastoret, P. P. 1988. Innocuité du virus recombinant vaccine-rage chez quelques especes non-cibles. In: Pastoret, P.P.; Brochier, B.; Thomas, I.; Blancou, J., eds. Vaccination to control rabies in foxes. Brussels: Office for official publications of the European Communities: 118–123.

Brochier, B.; Blancou, J.; Thomas, I.; Languet, B.; Artois, M.; Kieny, M. P.; Lecocq, J. P.; Costy, F.; Desmettre, P.; Chappuis, G.; Pastoret, P. P. 1989. Use of recombinant vaccinia-rabies glycoprotein virus for oral vaccination of wildlife against rabies: innocuity to several non-target bait consuming species. Journal of Wildlife Diseases 25: 540–547.

Brochier, B.; Languet, B.; Artois, M.; Zanker, S.; Guittre, C.; Blancou, J.; Chappuis, G.; Desmettre, P.; Pastoret, P. P. 1990. Efficacy of a baiting system for vaccinating foxes against rabies with vaccinia-rabies recombinant virus. Veterinary Record 127: 165–167.

Campbell, J. B. 1994. Oral rabies immunization of wildlife and dogs: challenges to the Americas. In: Rupprecht, C. E.; Dietzschold, B.; Koprowski, H., eds. Lyssaviruses. New York: Springer Verlag: 245–266.

Chappuis, G.; Kovalev, N. A. 1991. The rabies-vaccinia recombinant: from concept to application. In: Proceedings of the international conference on medical biotechnology, immunization and AIDS; May *1991; Leningrad, U.S.S.R. Leningrad: [publisher* unknown]: S6–2.

Charlton, K. M.; Artois, M.; Prevec, L.; Campbell, J. B.; Casey, G. A.; Wandeler, A. I.; Armstrong, J. 1992. Oral rabies vaccination of skunks and foxes with a recombinant human adenovirus vaccine. Archives of Virology 123: 169–179.

Cohn, J. P. 1986. Surprising cheetah genetics. Bioscience 36: 358–362.

Deshmukh, U. S.; Pal, R.; Talwar, G. P.; Gupta, S. K. 1993. Antibody response against epitopes on hGC mapped by monoclonal antibodies in women immunized with an anti-hCG vaccine and its implications for bioneutralization. Journal of Reproductive Immunology 25: 103–117.

Dowsett, K.F.; Tshewang, U.; Knott, L. M.; Jackson, A. E.; Trigg, T. E. 1993. Immunocastration of colts and immunospeying of fillies. Immunology and Cell Biology 71: 501–508.

Esposito, J. J.; Knight, J. C.; Shaddock, J. H.; Novembre, F. J.; Baer, G. M. 1988. Successful oral rabies vaccination of raccoons with raccoon poxvirus recombinants expressing rabies virus glycoprotein. Virology 165: 313–316.

Esposito, J. J.; Sumner, J. W.; Brown, D. R.; Ebert, J. W.; Shaddock, J. H.; He, B. X.; Dobbins, J. G.; Fekadu, M. 1992. Raccoon poxvirus rabies-glycoprotein recombinant oral vaccine for wildlife: further efficacy and safety studies and serosurvey for raccoon poxvirus. In: Brown, F.; Chanock, R. M.; Ginsberg, H.; Lerner, R. A., eds. Vaccines 92, modern approaches to new vaccines, including prevention of AIDS. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press: 321–329.

Fekadu, M.; Shaddock, J. H.; Sumner, J. W.; Sanderlin, D. W.; Knight, J. C.; Esposito, J. J.; Baer, G. M. 1991. Oral vaccination of skunks with raccoon poxvirus recombinants expressing the rabies glycoprotein of the nucleoprotein. Journal of Wildlife Diseases 27: 681–684.

Fenner, F.; Henderson, D. A.; Arita, I.; Jezek, Z.; Ladnyi, I. D. 1988. Smallpox and its eradication. Geneva: World Health Organization. 1,421 p.

Fletcher, W. O.; Creekmore, T. E.; Smith, M. S.; Nettles, V. F. 1990. A field trial to determine the feasibility of delivering oral vaccine to wild swine. Journal of Wildlife Diseases 26: 501–510.

Fu, Z. F.; Rupprecht, C. E.; Dietszschold, B.; Saikumar, P.; Niu, H. S.; Babka, I.; Wunner, W. H.; Koprowski, H. 1993. Oral vaccination of raccoons *(Procyon lotor)* with baculo-virus-expressed rabies virus glycoprotein. Vaccine 11: 925–928.

Grubb, G. S. 1991. Experimental methods of contraception (review). Current Opinion in Obstetrics and Gynecology 3: 491–495.

Haimovici, F.; Takahashi, K.; Anderson, D. J. 1992. Antifertility effects of antisperm cell-mediated immunity in mice. Journal of Reproductive Immunology 22: 281–298.

Hable, C. P.; Hamir, A. N.; Snyder, D. E.; Joyner, R.; French, J.; Nettles, V.; Hanlon, C. A.; Rupprecht, C. E. 1992. Prerequisites for oral immunization of free-ranging raccoons *(Procyon lotor)* with a recombinant rabies virus vaccine: study site ecology and bait system development. Journal of Wildlife Diseases 28: 54–79.

Hanlon, C. A.; Hayes, D. E.; Hamir, A. N.; Snyder, D. E.; Jenkins, S.; Hable, C. P.; Rupprecht, C. E. 1989. Proposed field evaluation of a rabies recombinant vaccine for raccoons *(Procyon lotor)*: Site selection, target species characteristics, and placebo baiting trials. Journal of Wildlife Diseases 25: 555–567.

Hanlon, C. A.; Buchanan, J. R.; Nelson, E. P.; Niu, H. S.; Diehl, D.; Rupprecht, C. E. 1993. A vaccinia-vectored rabies vaccine field trial: ante- and post-mortem biomarkers. Review Scientific and Technical Office International des Epizooties [Paris] 12: 99–107.

Hanlon, C. A.; Niezgoda, M.; Shankar, V.; Niu, H. S.; Koprowski, H.; Rupprecht, C. E. 1997. A recombinant vaccinia-rabies virus in the immunocompromised host: oral innocuity, progressive parenteral infection, and therapeutics. Vaccine 15: 140–148.

Hasegawa, A.; Koyoma, K.; Inoue, M.; Takemura, T.; Isojima, S. 1992. Antifertility effect of active immunization with ZP4 glycoprotein family of porcine zona pellucida in hamsters. Journal of Reproductive Immunology 22: 197–210.

Hierholzer, J. C. 1992. Adenoviruses in the immunocompromised host. Clinical Microbiology Review 5: 262–274.

Johnston, D. H.; Voight, D. R.; MacInnes, C. D.; Bachmann, P.; Lawson, K. F.; Rupprecht, C. E. 1988. An aerial baiting system for the distribution of *attenuated or recombinant rabies vaccines for foxes*, raccoons and skunks. Review of Infectious Diseases 10: S660–664.

Jones, G. R.; Sacco, A. G.; Subramanian, M. G.; Kurger, M.; Zhang, S.; Yurewitz, E. C.; Moghissi, K. S. 1992. Histology of ovaries of female rabbits immunized with deglycosylated zona pellucia macromolecules of pigs. Journal of Reproductive Fertility 95: 513–525.

Kirkpatrick, J. F.; Liu, I. M.; Turner, J. W.; Bernoco, M. 1991. Antigen recognition in feral mares previously immunized with porcine zona pellucidae. Journal of Reproductive Fertility 44: 321–325.

Kirkpatrick, J. F.; Liu, I. M.; Turner, J. W.; Naugle, R.; Keiper, R. 1992. Long-term effects of porcine zona pellucidae immunocontraception on ovarian function in feral horses *(Equus caballus)*. Journal of Reproductive Fertility 94: 437–444.

Ladd, A. 1993. Progress in the development of anti-LHRH vaccine (review). American Journal of Reproductive Immunology. 29: 189–194.

Lewis, J. C. 1968. use of poison baits to control rabies in Tennessee wildlife. Publ. Health Rep. 83. Knoxville, TN: Tennessee State Government: 68–74.

Linhart, S. B. 1964. Acceptance by wild foxes of certain baits for administering antifertility agents. New York Fish and Game Journal 11: 69–77.

Linhart, S. B.; Brusman, H. H.; Balser, D. S. 1968. Field evaluation of an antifertility agent, stilbesterol, for inhibiting coyote reproduction. Transactions of the North American Wildlife Conference 33: 316–327.

Liu, M. S.; Chan, K. M.; Lau, Y. F.; Lee, C. Y. 1990. Molecular cloning of an acrosomal sperm antigen gene and the production of its recombinant protein for immunocontraceptive vaccine. Molecular Reproduction and Development 25: 302–308.

Morell, V. 1993. Australian pest control by virus causes concern. Science 261: 683–684.

Moudgal, N. R.; Ravindranath, N.; Murthy, G. S.; Dighe, R. R.; Aravindan, G. R.; Martin, F. 1992. Long-term contraceptive efficacy of vaccine of ovine follicle-stimulating hormone in male bonnet monkeys *(Macaca radiata).* Journal of Reproductive Fertility 96: 91–102.

Nossal, G.J.V. 1989. Immunologic tolerance. In: Paul, W. E., ed. Fundamental immunology. New York: Raven Press: 571–586.

Parrish, C. R. 1994. The emergence and evoluation of canine parvovirus—an example of recent host range mutation. Seminars in Virology 5: 121–132.

Porter, W. F.; Mathews, N. E.; Underwood, H. B.; Sage, R. W.; Behrand, D. F. 1991. Social organization in deer: implications for localized management. Environmental Management 15: 809–814.

Rupprecht, C. E.; Johnston, D. H.; Dietzschold, B.; Koprowski, H. 1987. Development of an oral wildlife rabies vaccine: immunization of raccoons by a vaccinia-rabies glycoprotein recombinant virus and preliminary field baiting trials. In: Chanock, R. M.; Lerner, R. A.; Brown, F.; Ginsburg, H., eds. Vaccines 87, modern approaches to new vaccines. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press: 389–392.

Rupprecht, C. E.; Hanlon, C. A.; Hamir, A. N.; Koprowski, H. 1992a. Oral wildlife rabies vaccination: development of a recombinant virus vaccine. In: McCabe, R, ed. Transactions of the 57th North American Wildlife and Natural Resources Converence. Washington, DC: Wildlife Management Institute: 432–452.

Rupprecht, C. E.; Dietzschold, B.; Campbell, J. B.; Charlton, K. M.; Koprowski, H. 1992b. Consideration of inactivated rabies vaccines as oral immunogens of wild carnivores. Journal of Wildlife Diseasese 28: 629–635.

Rupprecht, C. E.; Hanlon, C. A.; Niezgoda, M.; Buchanan, J. R.; Diehl, D.; Koprowski, H. 1993. Recombinant rabies vaccines: efficacy assessment in free-ranging animals. Onderspoort Journal of Veterinary Research 60: 463–468.

Rupprecht, C. E.; Smith, J. S.; Krebs, J.; Niezgoda, M.; Childs, J. 1996. Current issues in rabies prevention: health dilemmas, public coffers, private interests. Public Health Reports 111: 400–407.

Talwar, G. P.; Singh, O.; Pal, R.; Chatterjee, N. 1992. Vaccines for control of fertility and hormone dependent cancers. International Journal of Immunopharmacology 14: 511–514.

Tartaglia, J.; Cox, W. I.; Taylor, J.; Perkus, M.; Riviere, M.; Neignier, B.; Paoleti, E. 1992a. Highly attenuated poxvirus vectors. AIDS Research in Human Retroviruses 8: 1445–1447.

Tartaglia, J.; Perkus, M. E.; Taylor, J.; Norton, E. K.; Audonnet, J. C.; Cox, W. I.; Davis, S. W.; VanDer Hoeven, J.; Meignier, B.; Riviere, M.; Languet, B.; Paoletti, E. 1992b. NYVAC: a highly attenuated strain of vaccinia virus. Virology 188: 217–232.

Tesarik, J.; Testart, J.; Nome, F. 1990. Effects of prolonged administration of anti-cumulus oophorus antibody on reproduction in mice. Journal of Reproductive Fertility 90: 605–610.

Tolson, N. D.; Charlton, K. M.; Stewart, R. B.; Campbell, J. B.; Wiktor, T. J. 1987. Immune response in skunks to a vaccinia virus recombinant *expressing the rabies virus glycoprotein.* Canadian Journal of Veterinary Research 52: 363–366.

Trewhella, W. J.; Harris, S.; Smith, G. C.; Nadian, A. K. 1991. A field trial evaluating bait uptake by an urban fox *(Vulpes vulpes)* population. Journal of Applied Ecology 28: 454–466.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service. 1992. Animal damage control. Factsheet. Hyattsville, MD: U.S. Department of Agriculture, Animal and Plant Health Inspection Service. 1 p.

U.S. Department of Agriculture, National Agricultural Statistics Service. April 1991. United States Department of Agriculture, Animal Damage Control. Washington, DC: U.S. Department of Agriculture, National Agricultural Statistics Service.

Vanage, G. R.; Garde, S. V.; Sheth, A. R.; Gopalkrishnan, K. 1992. Passive immunization against prostatic inhibin peptide as a male contrceptive. International Journal of Andrology 15: 114–126.

Westergaard, J. M. 1982. Measures applied in Denmark to control the rabies epizootic in 1977–1980. Comparative Immunology and Microbiology of Infectious Diseases 5: 383–387.

Wiktor, T. J.; MacFGarlan, R. I.; Dietzschold, B.; Rupprecht, C. E.; Wunner, W. H. 1985. Immunogenic properties of vaccinia recombinant virus expressing the rabies glycoprotein. Annales de l'Institute Pasteur/ Virologie 136E: 405–411.

Winkler, W. G.; Bogel, K. 1992. *Control of rabies in* wildlife. Scientific American 266: 86–92.

World Health Organization. 1993. Informal consultation on reproductive control of carnivores. Geneva: World Health Organization. 9 p.

Wynne–Edwards, V. C. 1964. Population control in animals. Scientific American 192: 2–8.

Zaunbrecher, K. I.; Smith, R. E. 1993. Neutering of feral cats as an alternative to eradication programs. Journal of the American Veterinary Medical Association 203: 449–452.