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Oral Immunization of Wildlife Against Rabies: Concept and First Field Experiments

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The possibility of immunizing carnivores against rabies with live attenuated vaccine administered by the oral route was raised by North American scientists in the 1960s. Subsequently, several American and European teams tested different vaccine strains in the laboratory for efficacy and safety and studied vaccine stabilization, vaccine delivery systems, bait acceptance by wild animals, and bait distribution schemes. The first field trial of a cloned SAD (Street Alabama Dufferin) strain in baits designed to immunize foxes orally was conducted in an Alpine valley in Switzerland in 1978. A population containing ~60% immune foxes at the valley entrance stopped the spread of the disease into untreated upper parts of the valley. The strategic use of oral vaccination of foxes in additional regions of Switzerland resulted in freedom from the zoonosis in four-fifths of the country.

The control of dog rabies by immunization has been successful since effective vaccines for veterinary use became available and were applied in mass vaccination campaigns. In many parts of the world, however, rabies remains endemic in populations of wild mammals. The idea of vaccinating susceptible free-living species suggests itself, but most early attempts at establishing herd immunities at a reasonable level in populations of wild carnivores failed [1]. Obviously this goal can be achieved only if self-vaccination is rendered attractive to the target species, e.g., by the incorporation of an oral vaccine into a bait. A breakthrough came when Baer, Debbie, and Abelseth [2, 3] discovered that some carnivores can be vaccinated orally with certain attenuated strains of rabies virus.

The goal of rabies control in wild animals should be local eradication or inhibition of spread into uninfected areas. The desired herd immunity can be established in a population only when the following technical criteria are met: (*1*) a vaccine that is safe and potent for field application; (*2*) a vaccine delivery system that assures vaccine contact with oral or intestinal mucosa; (*3*) an attractive vaccine vehicle or bait; and (*4*) an effective spatial and temporal pattern of bait distribution.

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Concepts, Models, and Predictions

The coexistence of rabies virus and its main host species is dependent on characteristics of the host population and rates of virus transmission. A rabid animal must, on average, infect one or more other individuals if the disease is to be maintained in the population [4, 5]. The question of how many animals in a population must be immune for the basic reproductive rate of infection to fall below this transmission threshold has been studied via mathematical models. Some models give rather encouraging answers [6, 7], whereas others make rabies eradication seem a nearly unattainable goal [8]. All of the models described so far in the literature take into account the dependence of the basic reproductive rate on population density, but most fail to consider that populations of foxes (the species targeted in our studies) are highly structured socially and live in structured habitats where disease transmission may not occur randomly [9]. Empiric data collected during dog vaccination campaigns suggest that rabies in dogs disappears when ~70% of the population is immune. However, since the social organization of dog populations is quite different from the behavior allowing infectious contacts in a fox population, this percentage should not necessarily be taken as the desired target figure.

Vaccines and Vaccine Virus Pathogenicity

A vaccine to be used for free-living wild animals should comply with a number of requirements (table 1). The first demonstration that foxes can be im-

Table 1. Requirements for a live attenuated rabies vaccine to be used for the immunization of free-living wild animals.

(*1*) Should orally immunize target animals.
(*2*) Should not be pathogenic for humans, for the target species, and for other species eating bait.
(*3*) Should not be excreted.
(*4*) Should not easily revert to higher pathogenicity.
(*5*) Should be free from pathogenic contamination.
(*6*) Should be storable.
(*7*) Should be stable at environmental temperatures for several days, but not for prolonged periods.
(*8*) Should be easy and inexpensive to produce.
(*9*) Should bear at least one genetic marker.

Table 2. Requirements for baits to be used as vehicles for a live attenuated rabies vaccine.

(*1*) Should be attractive to the target species.
(*2*) Should be eaten without being stored.
(*3*) Should be rejected by other species (including humans).
(*4*) Should reach a large proportion of the target population.
(*5*) Should not inactivate the vaccine.
(*6*) Should deliver the vaccine into the oral cavity.
(*7*) Should be able to incorporate a biologic marker (e.g., tetracycline).
(*8*) Should be easily available and inexpensive.

munized orally against rabies was made with the SAD (Street Alabama Dufferin) strain of rabies virus [2, 3, 10, 11]. SAD virus must infect oral and pharyngeal tissues to elicit an immune response. Although the virus is destroyed in the stomach [12], intact vaccine virus in the intestinal tract is capable of inducing immune responses [13]. Thus, to be effectively introduced into the intestine, the vaccine virus must be lyophilized and enterically coated. This procedure is complicated and expensive. For oral introduction, liquid vaccine must be enclosed in containers that, when ruptured, deliver it to mucous membranes in the oral cavity before it is swallowed.

SAD virus has some residual pathogenicity for a variety of rodent species tested [14–16]. It can also kill domestic animals and wild carnivores that have an impaired immune response, e.g., due to distemper in dogs ([17] and unpublished results from the Swiss Rabies Centre, Bern). So far, SAD virus has been reisolated from two animals. The isolate, injected into healthy animals, induced immunity and not disease (unpublished results, Swiss Rabies Centre). Neither laboratory studies nor field experiments have given any indication that the virus could be propagated within a population or community of wild animals [18].

Neither of the two other widely known attenuated rabies strains is a candidate for the immunization of wild animals. LEP (low egg passage) virus is too pathogenic (R. L. Parker, quoted in [1]), while HEP (high egg passage) virus is not efficacious enough [19] and easily reverts to higher pathogenicity [20].

It is possible to immunize an animal orally with inactivated vaccine. Highly concentrated antigen must be transported intact through the stomach and into the small intestine. A series of booster doses is required before the animal manifests a detectable immune response [13]. Oral immunization of wild animals with killed vaccine is clearly not the method of choice until new technologies allow the efficient transfer of swallowed antigens through the intestines to immunocompetent cells.

Baits and Vaccine Delivery Systems

The most important qualities that make a bait an effective vehicle for rabies vaccine are listed in table 2. Although a wide variety of baits have been tested in the field, only a few results have been published [21–24]. All bait types tested so far are attractive to various domestic and wild carnivores but often are also eaten by rodents [23], the animals most vulnerable to the residual pathogenicity of SAD virus. Most bait types also inactivate the vaccine virus. An exception are the chicken-egg baits suggested by Debbie [21]. These are well accepted by foxes but are then stored for prolonged periods. The problem of inactivation in meat and tallow baits can be solved by placing the vaccine in a container that is ruptured during bait uptake. However, another problem then arises: foxes often reject vaccine containers incorporated into otherwise texturally homogeneous baits. Considerable effort has therefore been expended in the development of a container that delivers the vaccine into the oral cavity before it is swallowed or rejected [25, 26].

Field Application of Baits

For maximal efficiency in a baiting campaign, the greatest possible proportion of the baits distributed should be picked up by the target species and not by competitors, and the greatest possible proportion of the target population should eat the bait before the vaccine has lost its potency. Thus, temporal and

spatial bait distribution strategies are required. The relation of bait location in a habitat to its subsequent discovery and acceptance by the target species has been studied for Swiss foxes [23]. No great differences were found in the rates of acceptance of baits placed within forests, along forest edges, and in fields and meadows. The findings might be entirely different, however, in other habitats with other competing food resources. For foxes living in permanent family territories, it is probably better to disperse the baits uniformly (rather than clustered at bait stations) so that every individual has access to a few baits [27]. Randomly distributed baits might be placed by hand on the ground [28] or spread from an airplane [24]. Whether caching the baits or promotion of their visibility is better again depends on characteristics of the target species, on the presence of food sources other than the baits, and on the presence of other animals competing for baits. An additional point to be considered is the residual pathogenicity of live attenuated virus. Baits should be laid out in a fashion minimizing the number of undesired contacts of humans with the vaccine.

Initial Field Applications of Vaccine Bait

Co-workers at the Swiss Rabies Centre and the Vertebrate Biology Unit of the Zoological Institute of the University of Bern have carried out a number of field and laboratory studies aimed at the development of a system of oral fox vaccination that is applicable in the field. Franz Steck directed this research until his untimely death in 1982. We thought that the cloned derivative of one of two SAD strains received from the Centers for Disease Control in Atlanta came closest to meeting safety and immunogenicity requirements [18, 28]. Chicken heads were chosen as baits. A vaccine container delivering the vaccine into the oral cavity of a fox chewing the bait was developed [26]. The immunizing capabilities of this system proved to be excellent in captive foxes. A 150-mg dose of tetracycline, injected into each bait, was sufficient to "label" the bones of bait consumers, thus allowing us to establish the percentage of foxes that had taken up at least one bait.

By 1978, the Rhone Valley, which is bounded on both sides by high mountain chains of the Swiss Alps, was threatened by the advance of fox rabies along Lake Geneva toward the valley entrance. This appeared to be an ideal epidemiologic situation for testing the efficacy of oral immunization of foxes. After consultation with the World Health Organization, permission was given by the Federal Veterinary Office, the Federal Office of Public Health, and the respective administrations of Canton Valais for an initial field trial in the lower Rhone Valley. During mid-October 1978, 4,050 vaccine baits were deposited over an area of 335 km² in the region of Martigny (Canton Valais). The disease did not cross the resulting barrier, consisting of a fox population of which ~60% were immune [28, 29]. For the maintenance of a level of herd immunity sufficient to inhibit the spread of rabies, the vaccination campaign was repeated in the spring and autumn of the following years. Since there was no direct proof that the spread of the disease was stopped by the presence of immune individuals, the experiment had to be repeated in other, similar situations, where an "immune barrier" could be created in the expected path of an epizootic wave. In no instance was the barrier crossed by the epizootic. The repetition of field trials in Alpine valleys finally freed the entire Swiss Alpine area from rabies.

In 1982 we had to decide on how to eradicate the disease in the Swiss midlands. High mountain ranges impeded the spread of the disease, and they greatly facilitated the strategic application of fox vaccination; this concept of natural and artificial barriers to disease also was applied to the rest of the country. We divided Switzerland into epidemiologic compartments delineated by natural and artificial obstacles to the spread of rabies, and we freed one compartment after another by immunizing the fox population (figure 1).

Today Switzerland is free of rabies except for an untreated area in the Jura Mountains bordering France and a small focus in Canton Aargau, south of the river Aare. We maintain an "immunization belt" along the borders of areas where rabies is still endemic.

Conclusions

The most important conclusions from 8 years of field application of rabies vaccine baits can be summarized as follows: By means of baits, it is possible to immunize enough free-living foxes to stop the spread of the disease into rabies-free areas and to eradicate the disease from an enzootic area. In areas where these goals were achieved, 50%–80% of all foxes were immunized. These figures are based on the demonstration of antibodies in serum and tetracy-

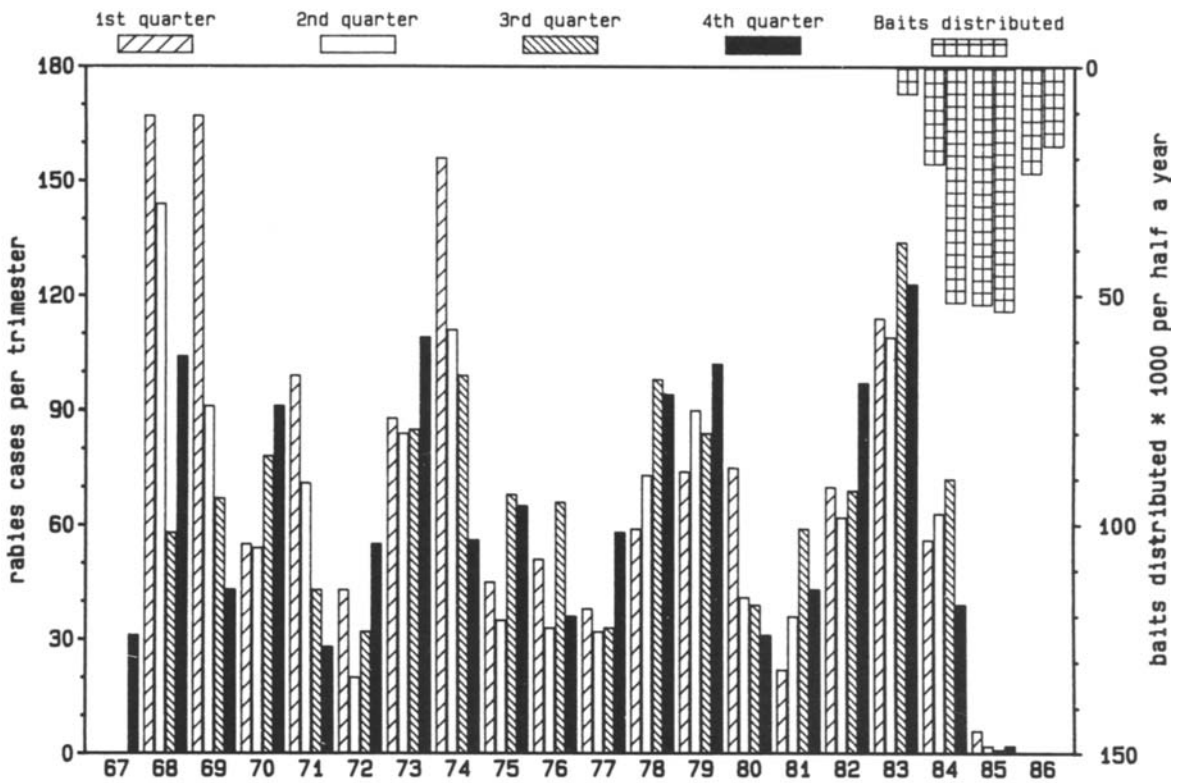

Figure 1. Number of diagnosed rabies cases per trimester in northeastern Switzerland (cantons Aargau [east], Appenzell Inner-Rhoden, Appenzell Ausser-Rhoden, St. Gallen, Thurgau, and Zurich; 5,500 km²) and number of vaccine baits distributed per 6 months. The first rabies case in the area was registered in November 1967 and the last in November 1985, 1 year after the first campaign of fox vaccination covering the total area. (The first vaccination campaign for part of the area was conducted in October 1983.)

cline in bone of killed foxes. In areas freed from fox rabies, rabies also disappears from all other species. The disease does not reappear spontaneously from an undetected reservoir after fox vaccination campaigns are discontinued. However, rabies may reinvade a fox population that is losing immunity as a result of population turnover.

The present success should not impede further efforts at improving methods of rabies control. We need oral immunization systems for other important hosts, such as free-roaming domestic dogs in the developing world and possibly raccoons, skunks, and mongooses. In addition, we need other vaccines with less residual pathogenicity. Some promising results with recombinant vaccinia virus were recently published [30]. Finally, we may need artificial baits (like those already applied in the Federal Republic of Germany) that are better suited for industrial production and storage.

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