THE ORAL DELIVERY OF RABIES VACCINES TO DOGS

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Abstract

In most developing countries the dog remains the major transmission vector of rabies to man, despite the widespread use of parenteral vaccination. Parenteral vaccination does not, in general, achieve population immunity levels high enough to inhibit rabies transmission within the dog population. Following the demonstration that oral vaccination induced levels of population immunity sufficient to interrupt the epidemiological cycle of fox rabies in Europe, research has aimed at the development of this approach for dog rabies control.

Oral vaccines are likely to be easier to administer and culturally better tolerated, and therefore well adapted to overcome the logistical problems hampering rabies control in developing countries. Among several proposed rabies vaccine candidates, the attenuated rabies vaccine strain SAG-2 has been shown to induce a protective immune response to rabies in dogs when delivered by bait.

Oral live vaccines must meet high safety standards due to the risk associated with the dissemination of those replicating antigens in young and/or immunodepressed individuals. SAG-2 has confirmed its safety through the absence of clinical signs, dissemination and excretion in several carnivorous target and an important number of non-target species including non-human primates.

Present field research, carried out using a placebo bait specifically designed for the oral delivery of SAG-2 to dogs, is aiming at confirming the hypothesis according to which oral vaccination, alone or as a possible adjunct to injectable rabies vaccines, might increase vaccine coverage by immunising poorly supervised dogs which are both often inaccessible to parenteral vaccination and a high-risk group for rabies transmission to man.

Introduction

In developing countries, canine rabies control has achieved only limited success despite extensive effort and expense. One often suggested reason for this failure is that dog vaccination levels sufficient to break the dog-to-dog transmission cycles are rarely reached (B6gel et al. 1982; Perry 1993) or cannot be maintained, so that transmission is inevitably re-
established (El Hicheri 1993). It is often suggested that problems such as inadequate logistics, insufficient community participation or inaccessibility of dogs are responsible for this failure and it is hypothesised that these could be overcome with oral vaccines for dogs (see "Evolution of WHO recommendations on oral immunisation of dogs against rabies" page 145). Rabies control by oral immunisation is possible and has been clearly demonstrated with respect to fox rabies in Europe (Wandeler et al. 1988; Brochier et al. 1991; Aubert et al. 1993). The techniques developed to control wildlife rabies in Europe and North America and their delivery does however require adaptation to be successful in the dog. The approach chosen by our laboratory was the development of the attenuated vaccine strain SAG-2. Until now, only live vaccines have been shown to confer an adequate protective, systemic immune response against rabies in dogs at concentrations conducive to an economical vaccine use (Rupprecht et al. 1992). These vaccines have the capacity to replicate locally in the vaccinated animal and in this way the viral antigens are presented to the host's immune system more effectively than would be the case for non-replicating agents (O'Hagan 1992). However, due to the risk of mutations during replication and the proximity of dogs to humans, vaccines intended for the oral immunisation of dogs have to meet high safety standards. Safety recommendations issued by the YMO specify that vaccines should be innocuous for humans, for the target species including very young dogs and for non-target species likely to be attracted by the bait delivery system (WHO 1995). They should not be excreted, as excretion may be indicative of local replication and consequently an increased risk of mutation, reversion to pathogenicity and transmission.

It is assumed that effective rabies control in countries with canine rabies requires the immunisation of a large proportion of the dog population (about 70 percent) in order to reduce the contact rate between infectious and susceptible dogs to a level too low to sustain rabies transmission within the population (Beran 1991). As this level of vaccine coverage is difficult to obtain and maintain, targeting rabies control measures is thought to increase the success of such measures and render them more economic and viable (Perry 1993). Studies are currently being undertaken to analyse the so-called high-risk section of the dog population (in terms of exposure to and transmission of rabies) which are composed of true feral and totally or partly unrestricted, owned dogs. These dogs, characterised by minimal supervision and high dog-to-dog contact rates (Table 1) are likely to be less accessible to parenteral vaccination and to constitute the main target for oral vaccination (Matter 1993). The field trials summarised in this document, using a freeze-dried placebo dog bait, were recently conducted in two rural villages in Tunisia in order to determine the accessibility of certain subpopulations of dogs to this method, the risk of human exposure to vaccine virus and the logistical efforts associated with bait distribution (Ben Youssef et al. unpublished data).

The vaccine candidate SAG-2

SAG-2 is a live rabies virus that was selected from a pool of SAD Bern variants by monoclonal antibodies directed against the external glycoprotein of the rabies virus (Lafay et al. 1994). The resulting variant differs from the parental strain SAD (Bern) by two
nucleotides in a triplet coding for an aminoacid (Table 2) in a region of the glycoprotein associated with the virulence of rabies viruses (Coulon et al. 1983). SAG-2 has lost the residual pathogenicity for mice by the oral, intramuscular and intracerebral route that characterises the parent strain SAD Bern (Lafay et al. 1994).

Table 1. Classification of dogs based on their dependency and restriction

<table>
<thead>
<tr>
<th>Full restriction</th>
<th>Semi-restriction</th>
<th>No restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs physically</td>
<td>Dog with access to</td>
<td>Dog with free access</td>
</tr>
<tr>
<td>separated from rest</td>
<td>the rest of the dog</td>
<td>to the dog population</td>
</tr>
<tr>
<td>of the dog population</td>
<td>population</td>
<td>at all times</td>
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</table>

Full dependency
Dog given all of its essential needs intentionally by humans

Semi-dependency
Dog is given a proportion of its essential needs intentionally by humans

No dependency
Dog is given none of its essential needs intentionally by humans

Inoculation of dogs, baboons and over 30 mammalian and avian animal species by the oral route produced no adverse effect and no evidence of virus excretion could be detected (E. Masson et al., in submission to Vaccine; M. Fekadu et al, J. Bingham et al., F. Chapparo et al., unpublished data). Since 1992, the strain has been successfully used for fox rabies control in Europe (Masson et al. submitted for publication) without any report of negative impacts on the environment.

The vaccine delivery system

The vaccine delivery system developed to vaccinate dogs by the oral route with SAG-2 is composed of two main parts: a freeze-dried central core containing the vaccine and a thin layer of bait matrix attractive to dogs.
The dimensions of the internal unit are approximately 1.5 (H) x 3 (L) x 2.5 (W) cm. Contact with small amounts of aqueous solution leads to the re-hydration of the unit within seconds. This is of importance because, in order to be active, the vaccine virus needs to be reconstituted in the saliva of the dog. Since dogs swallow food generally without chewing, only small quantities of saliva are available. The reconstitution of the vaccine is promoted by the adhesive character of the central unit due to adsorption of bait particles to the mucosa. This characteristic induces a licking reflex in the dog, promoting saliva secretion and consequently bait disintegration.

The freeze-dried unit is coated by a matrix which is attractive for dogs and protects the core unit against minor shocks and humidity. It is composed of materials of animal origin, artificial taste enhancers, and a synthetic polymer responsible for impermeability and mechanical stability.

Table 2: Differences between SAD Bern and SAG-2 in codon and aminoacid sequence 333 of the rabies glycoprotein.

<table>
<thead>
<tr>
<th>Virus Strain</th>
<th>Nucleotide Sequence</th>
<th>Aminoacid in Position 333</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAD Bern</td>
<td>AGA</td>
<td>Arginine</td>
</tr>
<tr>
<td>SAG2</td>
<td>GAA</td>
<td>Glutamic Acid</td>
</tr>
</tbody>
</table>

Efficacy of the vaccine delivery system in dogs.

Two groups of four adult laboratory beagles, from whom food was withheld 24 hours prior to the experiment, were offered a single bait containing either $10^{9.0}$ median tissue culture infectious doses (TCID$_{50}$) or $10^{8.0}$TCID$_{50}$ of SAG-2. Two dogs were not vaccinated and kept as challenge controls. Seven of eight vaccinated animals were interested in the bait and consumed it within a period of 2 min. 16 sec to 11 min. 55 sec. One dog of the group receiving the bait with the higher vaccine titre consumed only half of its bait. Twenty-nine days after treatment, all vaccinated and unvaccinated dogs were challenged with a lethal dose of canine street virus injected into the temporal muscle. The two control animals died of rabies as expected, 19 and 26 days later. One animal in each vaccination group also succumbed to rabies 14 and 16 days following challenge (Table 3). Both animals had consumed an entire bait. The shortened incubation period in the vaccinated dogs could indicate the occurrence of an early death phenomenon (Sikes et al. 1971, Blancou et al. 1980), but the small number of animals renders an interpretation of this observation difficult. None of the eight vaccinated and the two control dogs presented detectable levels of
seroneutralising antibodies before vaccination or before challenge on day 29. Despite the fact that neutralising antibody titres were undetectable prior to challenge, three out of four dogs which received a bait containing either $10^{9.0}$ TCID$_{50}$ or $10^{8.0}$ TCID$_{50}$ were protected against a lethal rabies virus challenge. The difference between the vaccination group and the control group is significant when considering, that the challenge virus concentration corresponds to at least 1 LD100 for dogs (100 percent of controls succumbed) (M. Aubert and C. Schumacher et al., unpublished data).

Table 3  Efficacy of a single freeze-dried bait containing SAG-2 in dogs

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Vaccine conc. per bait</th>
<th>YNA* on day of challenge</th>
<th>% Challenge mortality**</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 dogs</td>
<td>$10^9$ TCID$_{50}$</td>
<td>0/4</td>
<td>25%</td>
</tr>
<tr>
<td>4 dogs</td>
<td>$10^9$ TCID$_{50}$</td>
<td>0/4</td>
<td>25%</td>
</tr>
<tr>
<td>2 controls</td>
<td>none</td>
<td>0/2</td>
<td>100%</td>
</tr>
</tbody>
</table>

* VNA: Virus Neutralising Antibodies
* Intramuscular challenge with $10^{3.64}$ median mouse intracerebral lethal doses (MICLD$_{50}$) of lethal canine street rabies virus 29 days following vaccination.

Modes of bait distribution

The following studies were carried out in two different test sites in an rural area in the proximity of Tunis. The data presented hereunder are estimates based on a preliminary analysis. The total dog population in either villages accounted for approximately 300 dogs, only 5-15 percent of which were considered unowned and free all the time. The majority of dogs were used for guarding and were tied up on the property.

1. Bait distribution at mobile vaccination centres

Following an information campaign, baits (placebo DBL-2) containing the serum-marker sulfadimethoxine (SDM were handed out to dog owners (one bait per dog) at a mobile vaccination centre with precise instruction for use. The next day each household of the village was visited and the bait consumption determined by questionnaire survey and by serum analysis to reveal the presence of serum marker. According to either analysis, over 90 percent of the owned dogs had consumed a bait and 5 to 15- percent of these dogs were
considered inaccessible for parenteral vaccination. Truly unowned dogs did not receive baits. The logistical effort of the method did not exceed that normally spend for parenteral vaccination campaigns. Due to good compliance with the instructions, human exposure was minimal (Table 4).

2. **Door-to-door distribution**

Following an information campaign, each household of village 2 was visited and a bait (placebo DBL-2) was given to dogs present at the time of the visit. Consumption was determined by direct observation. Sixty-five percent of all owned dogs accepted a bait. The proportion of these dogs that was inaccessible to parenteral vaccination was not determined, but can be considered low due to the fact that unapproachable dogs, freely moving on their territory, frequently left upon arrival of the vaccination team. The logistical effort of the distribution method was very important. Bait acceptance was low compared to the previous distribution mode and might be related to the presence of the vaccination team on the premises. However, for the same reason, the risk of human contamination was minimal (Table 4).

*Table 4.* Vaccine coverage, risk of human contamination and logistical efforts of oral vaccination compared to parenteral vaccination

<table>
<thead>
<tr>
<th>Bait Distribution Mode</th>
<th>Theoretical Vaccine Coverage</th>
<th>Risk of Human Exposure to Vaccine Virus</th>
<th>Logistical Effort &amp; Vaccine Cost*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Dog Population</td>
<td>High Risk Population</td>
<td></td>
</tr>
<tr>
<td>Central Point</td>
<td>81%</td>
<td>8%</td>
<td>low</td>
</tr>
<tr>
<td>Door to Door</td>
<td>59%</td>
<td>&lt;8%</td>
<td>none observed</td>
</tr>
<tr>
<td>WIM**</td>
<td>25%</td>
<td>6%</td>
<td>high</td>
</tr>
</tbody>
</table>

* Compared to parenteral vaccination campaigns
** WIM = Wildlife immunisation model

3. **Wildlife immunisation model**

Following an information campaign, over 1000 baits (placebo DBL-2) containing the serum marker, SDM, were distributed along roadsides and around village 2 at a density of 4.7 baits per dog and left there over night. The next day, each village household was visited and a
blood sample was drawn from every accessible owned dog. Free-roaming dogs were bled after having been anaesthetised by using a dart blow pipe. Bait acceptance was assessed by SDM analysis on sera collected.

Twenty-three percent of the owned dogs, of which 7.5 percent were considered inaccessible to parenteral vaccination had consumed a bait. Forty percent of the dogs with unknown ownership status (the total dog population comprised 10-15 percent of the dogs with uncertain ownership status) also consumed a bait. The logistical effort, bait costs and the risk of human contamination were considered high in this case (Table 4).

**Discussion**

The live modified rabies vaccine strain SAG-2 has fulfilled most of the WHO recommendations for efficacy and safety and is due to undergo field evaluation. A suitable vaccine delivery system for carnivores, a freeze dried bait suitable for the delivery of the SAG-2 vaccine into the oral cavity of dogs is under development. The bait is well accepted by owned and unowned dogs. Consumption of a single vaccine laden bait confers protection against a severe street virus challenge to approximately 80 percent of animals vaccinated in the laboratory.

Bait distribution in two Tunisian field sites according to the central point and door-to-door distribution mode reached primarily owned dogs accessible to parenteral vaccination and to a lesser extent, owned dogs inaccessible to parenteral vaccination. Bait distribution according to the wildlife immunisation model reaches primarily unrestricted owned dogs and unowned dogs which together constitute the high risk group in terms of rabies transmission and are largely inaccessible to vaccination. However, the risk of human contamination, especially of children, and the costs of this method are unacceptable. None of the distribution modes reaches more than 10 percent of the high risk dog population, but this might nevertheless be sufficient. The solution could be provided by combining parenteral vaccination of accessible owned dogs with the distribution of baits to owners who could not bring their dog to the vaccination centre. If necessary, unowned dogs could additionally be targeted by the distribution of baits in delimited areas, where unowned and free-roaming owned dogs share resources. Used in combination with parenteral vaccination of dogs, oral vaccination is unlikely to reduce the costs of rabies control in the short run, but the increase of vaccination coverage in the vector population could, as witnessed in some European countries practising oral vaccination of foxes, lead to rabies elimination.

**References**


