REPORT OF
THE FOURTH WHO
CONSULTATION ON
ORAL IMMUNIZATION
OF DOGS AGAINST
Rabies

Geneva, 14-15 June 1993
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1. **INTRODUCTION**

Dr F.-X. Meslin, Chief, Veterinary Public Health unit, welcomed the participants on behalf of Dr Hiroshi Nakajima, Director-General of the World Health Organization.

The objectives of the Consultation were as follows:

1. to review the results of experiments carried out since the 3rd Consultation held in July 1992, attention being especially given to results on safety of candidate vaccines for non-target species;

2. to identify the segments of the dog population as well as the vaccine-bait delivery systems which would, in most situations, lead to disease elimination and ensure maximum safety;

3. to discuss formulation of dog baits; and

4. to update conclusions and recommendations with special reference to the WHO protocol for assessing efficacy and safety of candidate vaccines in target species and safety in non-target species.

Dr C.E. Rupprecht was elected Chairman of the Consultation and Dr S. Linhart was nominated Rapporteur.

2. **PROGRESS MADE IN THE TESTING OF ORAL VACCINE EFFICACY IN DOGS (AND OTHER CARNIVORES)**

2.1 **Vaccinia rabies glycoprotein recombinant (VRG)**

A previous experiment in dogs with a VRG bait containing the current field dose used for oral vaccination of foxes in Europe (i.e. \(10^{8.5} \text{TCID}_{50}\) in 3 ml) showed that dogs having received 2 or 3 baits were protected against challenge 10 months after first vaccination (see Report of the 3rd Consultation on Oral Vaccination of Dogs - Document WHO/Rab.Res./92.38). The same experiment was conducted again on three groups of dogs (i.e. 5 dogs, 2 dogs, 2 dogs) which received respectively 1, 2 and 3 baits at 24-hour intervals. A challenge was performed 13 months later. All dogs, regardless of the number of baits ingested, were protected (5 of 5 controls died of rabies).

Initially VRG was produced on Vero cells. In Europe it is now produced on BHK cells and it has been shown to be equally potent for both foxes and dogs.

2.2 **Human Adenovirus 5 (HAV5) rabies glycoprotein recombinants**

HAV5 rabies glycoprotein recombinants (Ad5-\(8E3-1\)C1, Ad5-\(8E3-RG4\), Ad5-\(8E3-RG1.3\), Ad5-\(8E1-RG\)) immunize skunks by the oral route when given directly into the oral cavity. Ad5-\(8E3-RG1\) also immunizes foxes, dogs, and raccoons (other
constructs are not tested as yet). The immunizing properties of Ad5-8E3-RG1 are much poorer, when it is administered in baits. Some of the difficulties of the application in baits may be overcome, when the more potent Ad5-8E3-RG1.3 is used. Preexisting immunity against HAV5 interferes with Ad5-RG1 immunization. In domestic carnivores naturally acquired antibodies to HAV5 are very rare.

2.3 Modified live vaccines

2.3.1 SAD B19

Fourteen local dogs were orally vaccinated in captivity in the Etkik Institute (Ankara, Turkey) in November 1992 with the SAD B19 strain using a vaccine virus titre of $2 \times 10^8$ FFU. Three dogs had pre-existing antibody titres, of which two demonstrated an anamnestic response following vaccination. Eleven dogs seroconverted.

All dogs withstood a challenge on 8 March 1993 ($3 \times 10^5$ MIC LD$_{50}$/ml NYC virus), but of 10 control dogs only four died.

In Istanbul, free-roaming dogs were first offered chicken head baits containing SAD B19 in plastic packets. If this bait was not eaten, dogs were then offered a Köfte (minced lamb) bait containing an identical vaccine sachet. A total of 134 dogs were offered baits over a 4-day period. Of these, five refused either bait, one third accepted the chicken head bait, and the remainder took the Köfte bait.

Forms were developed to provide a standardized procedure for data collection when programmes combining oral and parenteral vaccination are carried out.

2.3.2 SAG

Efficacy tests of SAG have been performed on dogs: 2 groups of 5 dogs were administered $10^7$ PFU or $10^8$ PFU per os. All 10 dogs were protected against a challenge that killed 4 out of 5 controls. Similarly, all experimental foxes (16) were protected with $10^7$ PFU (or more) administered per os against a challenge that killed all controls. All vaccinated dogs (10) and foxes (28) seroconverted.

2.4 Inactivated vaccines

Considerations of the specific use of oral rabies vaccines intended for dogs have become especially complicated due to the human-animal bond and the increasing spectre of hosts with altered immunocompetence; these may include various congenital, infectious, environmental or iatrogenic aetiologies of immunodeficiency, in both public health and veterinary sectors. Thus, the use of live vaccines is discouraged when the risk of unintentional exposure to severely immunocompromised populations is deemed high, because of the chance of enhanced viral replication, altered tropism or untoward adverse events; conversely, inactivated vaccines do not appear to represent a danger to immunocompromised individuals. While it is possible to induce non-tolerogenic, protective immunity against rabies with ingested, traditionally-purified antigens this approach is impractical considering the costs involved. Alternatively, viral expression vectors, such as the baculovirus system, generate large amounts of individual viral proteins (e.g. 50 mg/l) and may potentially provide an economical source of a subunit vaccine devoid of many of the drawbacks typically associated with self-replicating organisms. To this end, a baculovirus-rabies recombinant vaccine was
developed which expressed rabies glycoprotein in insect cell culture which was
antigenically similar to authentic ERA protein; this vaccine induced rabies VNA
and protected raccoons against severe street virus challenge when administered
per os in two, individual liquid boluses of 100 µg each. Given this initial
finding in a relevant carnivore species, it was of interest to extend the
observation to dogs. Specifically, it was asked if a single oral administration
of baculovirus vaccine could prime or boost the rabies VNA response in adult
animals that were either naive or had previously been exposed to rabies, and
survived. Yet, neither a primary induction nor an anamnestic VNA result was
found some 14 days post-vaccination. Possibly, a single vaccine administration
was inadequate, coupled with the use of rather aged dogs. Additional research,
including potency and stability tests, compatibility with adjuvants and the
feasibility of producing a chimeric (e.g. N + C) recombinant, is in progress.
Furthermore, an in-depth analysis of alternative antigen delivery systems (e.g.
microspheres) for oral vaccination, which promote strong and sustained systemic
immune responses, needs to be undertaken. Future results require careful
assessment before the concept of an inactivated rabies oral immunogen for dogs is
abandoned as the next obvious generation of vaccine. In lieu of an alternative,
several candidate attenuated or recombinant rabies vaccines appear to offer safe,
efficacious and economical solutions for the oral immunization of immunocompetent
hosts.

3. SAFETY TESTS IN NON-TARGET SPECIES AND IMMUNOCOMPROMISED ANIMAL MODELS

3.1 In non-target species

3.1.1 SAG2

In so far as SAG2 has been tested for innocuity on several species
according to the protocol recommended by WHO, no lesion, or specific mortality
was observed in the inoculated animals. No virus has been re-isolated from the
brain of these animals. The table set out as Annex 2 summarizes these findings
and indicates for each non-target species the route or routes of vaccine
administration as well as the dose and duration of observation.

3.1.2 SAD B19

On 1 September 1992, 10 chimpanzees aged between 3½ and 8 years were orally
vaccinated with the SAD B19 vaccine virus strain. The dose was 1 ml, the virus
titre $1.5 \times 10^6$ FFU/ml. Observation time of the chimpanzees was over 90 days.
Clinical signs of rabies were not observed during this observation period.

3.1.3 Human Adeno virus-5 recombinants (HAV5-RG)

HAV5-RG’s are very stable. Vaccine virus is excreted by target and non-
target species and a certain degree of environmental contamination is inevitable
(e.g. faeces, self-contamination, etc.). HAV5-RG’s cause lesions in lungs of
small rodents, when given intra-nasally in very high doses ($>10^{10}$ TCID$_{50}$). This
residual pathogenicity is considered to be insignificant for oral exposures. The
majority of the previously stated safety concerns may be irrelevant when HAV5-RGs
are considered for field use.
3.2 Safety tests in immunocompromised animals

3.2.1 VRG

a) Severe combined immunodeficient (SCID) mice

After the accumulation of a large body of vaccine safety data on a vaccinia-rabies glycoprotein (VRG) recombinant virus in over 50 vertebrate species (in which no adverse vaccine-related lesions or effects could be identified), a more sensitive animal model was desired for the description of potential adverse effects in the immunocompromised host. In the SCID mouse, an autosomal recessive gene defect precludes the development of functional B and T cells thereby resulting in a profound immunodeficient state.

Following intramuscular administration of 30 μl of a parenteral strain (thymidine kinase+) of vaccinia virus (10^8.2 PFU/ml) in SCID mice, disseminated lesions were observed within one week, at peripheral sites such as the tail, toes, nose, mouth, ears and genitalia, which progressed rapidly and resulted in weight loss and death by day 21. Comparatively, although 30 μl of VRG virus (10^9 PFU/ml) resulted in disseminated lesions consistent with vaccinia, the attenuated nature of the VRG (thymidine kinase-) was demonstrated by the delay in the development of initial lesions to between 12 and 60 days, markedly slower lesion progression, and less rapid loss of body weight. Similar results were observed by other parenteral routes, such as intraperitoneal, intracranial and intradermal.

In summary, the attenuated nature of the thymidine-kinase negative VRG virus (in comparison to its parenteral vaccinia virus) in SCID mice was consistent with findings in immunocompetent mice and adverse effects of vaccinia and VRG were demonstrated to be dose and route dependent.

Thus, the SCID model is useful for comparison of various relatively apathogenic vaccine viruses (that may produce no adverse effects in the typical immunocompetent host) with respect to potential virulence in the immunocompromised or immunodeficient host.

b) Domestic cats

During the first North American VRG vaccine field efficacy trial in southern New Jersey (USA), up to 20% of apparently unowned, or poorly supervised, free-ranging domestic cats in the vaccination area had evidence of bait contact. Given the potential higher risk of this population for infection with Feline Leukaemia Virus (FeLV), Feline Infectious Peritonitis (FIP) and Feline Immunodeficiency Virus (FIV), and the close overall contact of domestic species with humans, the public health impact of a potentially prolonged, or more extensive VRG viral infection makes it prudent to evaluate the VRG vaccine in these potentially viral-immunosuppressed animals.

In two related studies, domestic cats with and without potentially immuno-suppressive feline viral infections received VRG virus by the oral (1 ml of 10^8 PFU/ml) and intradermal (100 μl of 10^9 PFU/ml per site) routes. Observations included the description of lesions at the site of VRG virus administration with regard to size, induration and swelling. There were significant differences between control sites (intradermal PBS or dH₂O) and VRG sites within animals with respect to lesion formation, size, redness and swelling. However, there were no differences in the development and regression of mild intradermal lesions at the
sites of VRG inoculation, regardless of feline viral infection status (i.e. healthy control cats versus FeLV or FIV positive cats).

No lesions resulted from oral administration of VRG virus. In addition to these preliminary clinical observations, no differences in body weight, body temperature, PCV, TS, or clinical attitude were observed during the three-week observation period of 21 cats in the FeLV study (prior to necropsy on day 21) nor during the initial seven-day observation period of the on-going FIV study (9 cats). Virus isolation and PCR detection of VRG from oral and anal swabs, and tissues, CD4/CD8 ratio determinations, white blood cell counts and differentials, serology (anti-rabies and anti-vaccinia) are in progress.

3.2.2 SAG2

The pathogenicity of the modified live rabies vaccine strain SAG-2 for the SCID mouse was determined. SAG-2 was administered at two concentrations (10^7.5, 10^9 TCID50/ml) by oral (0.02 ml), intramuscular (0.05 ml) and intracerebral (0.03 ml) routes. Ninety-five per cent of all immunodeficient individuals inoculated by the two latter routes succumbed to rabies. Severity and duration of clinical signs correlated with vaccine dosage and were more pronounced after intracerebral than intramuscular inoculation.

Whatever the concentration (i.e. 10^7.5 and 10^9 TCID50/ml) all SCID mice inoculated per os and all adult, outbred and immunocompetent ICR control mice (White Swiss) which were administered the vaccine parenterally or orally, survived the observation period without showing clinical signs. These results confirm that SAG2 does not lead to rabies infection unless administered parenterally to mice with severely impaired or immature immune systems.

4. TESTING BAITS AND BAIT DELIVERY SYSTEMS

4.1 Bait formulation and baiting trials

4.1.1 Concepts in bait formulation

An important precondition to the initiation of evaluating bait distribution techniques in the field is the availability of a bait well accepted by the target species under field conditions. A bait which is poorly accepted by dogs, even if all other requirements are well fulfilled, has no use for oral mass vaccination. A bait is defined among other parameters by its size, colour, shape, odour, caste, and texture. A further parameter could be called harmony which stands for the balance of all these parameters together.

The odour of a bait should attract dogs and at the same time trigger a food uptake desire in the dog. For this reason, the use of attractants which trigger a different behavioural state (e.g. mating behaviour) is not recommended. The size and shape of a bait should facilitate their handling by dogs. On some occasions it was observed that dogs had problems taking rather flat fox baits tested in Tunisia. Texture is an important parameter of a bait as a vaccine delivery device. If the bait material is too soft, the animal may recognize the hard vaccine container as an alien component and spit it out. A very resistant bait matrix, on the other hand, may be too hard to be chewed by a puppy. Harmony may be important for the decision of a dog to consume a bait. Lack of harmony may prevent a dog from consuming a bait, even if individual parameters would have been accepted.
It is not possible to establish the chain of decisions made by a dog without controlled behavioural studies. However, while doing bait acceptance evaluation on owned dogs in Tunisia, certain behavioural sequences were observed which were almost constant for dogs approaching a bait. In general, a dog first investigates the bait by sniffing and licking it. The dog then takes up the bait by means of the incisors, and finally consumes it. Interruption and bait refusal was possible at any step of this sequence, but it most often occurred after smelling or licking.

Quite often, dog owners succeed in making dogs accept a bait by offering by hand or by mixing the bait into the dog's food. A dog is generally less critical to unknown pieces of food if it is offered by the person who usually feeds it. In addition, the way of offering a bait to a dog, may trigger a bait uptake regardless of the nature of the bait if given in the presence of or by the owner himself.

The effects are enhanced if the bait is mixed with food to which the dog is accustomed.

For free-roaming owned dogs and ownerless dogs no similar factors can support the dog's decision to accept the bait. The concept of harmony becomes especially important when baits are used to vaccinate dogs with no close relation to an owner. Yet, oral vaccination was assumed to be a supplementary method to reach exactly this part of the dog population (i.e. ownerless dogs and free-roaming owned dogs).

4.1.2 Current status of dog baiting trials

Recent tests of baits for administering oral vaccines to dogs have borrowed from earlier wildlife studies, and the few baits evaluated so far have largely been those previously developed for red foxes in Europe and Canada and for raccoons in the United States. Only a few field tests incorporated a vaccine container and a placebo vaccine into baits in a manner similar to that envisaged for an actual oral rabies vaccine. Moreover, none of the baits contained a systemic biomarker nor was the dog population subsequently sampled to determine the percentage of dogs that actually ingested the placebo vaccine. Thus, while the studies to date have provided much useful information, definitive data indicative of the probable success of oral vaccination are still lacking.

Chicken baits have often provided the best results, but their widespread use as a vehicle for vaccine application may be limited. No manufactured bait tested to date has shown uniformly high rates of acceptance, and dogs seem adept at distinguishing between bait material and vaccine container. Observations in rural Mexico revealed that dogs were generally cautious about approaching baits and consumed them slowly and deliberately. Dogs sometimes separated ampoules from baits, ampoules were sometimes poorly penetrated, and varying amounts of placebo vaccine were either retained within capsules or lost onto the ground.

Chicken baits and fishmeal baits without vaccine were tested in a waste disposal site visited by free-roaming owned and ownerless dogs in Northern Tunisia in order to compare their value as vehicles for the oral application of antirabies vaccine for free-roaming dogs. No vaccine was used. Bait acceptance was measured by the tracking-station method. The baits were available for 36 hours and replaced periodically. Some dogs came to the study site at any time of the day, and a large number of dogs (30 or more) was only observed during a given period of time. The number of chicken-head baits probably picked up by dogs was more than seven times greater than the number of fishmeal baits. Fishmeal baits
were characterized by a certain attractiveness for cats. Chicken-heads are well accepted by free-roaming dogs, are inexpensive, locally available, unattractive to humans, easy to handle and to store in large quantities.

4.2 Evaluating bait delivery systems

A basic study concept for the development of a research project for the field evaluation of several vaccine-bait delivery systems has been elaborated (WHO/Rab.Res./92.38 - Report of the 3rd Consultation on Oral Vaccination of Dogs). Its use should facilitate the comparison of different vaccine delivery models.

At least three bait distribution techniques deserve to be evaluated:

(a) Similarly, the "parenteral vaccination model" vaccine-baits can be given to dog owners in mobile distribution centres or may be fed to dogs directly by door-to-door distribution.

(b) Bait distribution can be performed according to the "Wildlife Immunization Method" (WIM). The distribution of the bait can be made homogeneously over the entire vaccination area or baits can be deposited following lines (e.g. linear distribution along roads) or can be deposited in clusters ("feeding spots").

(c) Baits can be fed directly to free-roaming dogs ("hand-out model").

In order to complete the data on accessibility of dogs to oral immunization, a first evaluation should involve two of the distribution techniques mentioned above.

- bait distribution according to the parenteral vaccination model (to dog owners and to dogs by door-to-door baiting);

- bait distribution according to WIM.

The target sub-population of the first method is owned dogs. The second technique (WIM) is directed at ownerless dogs or any free-roaming dog not accessible by another method (parenteral vaccination or bait distribution to owned dogs). A pre-condition to the initiation of these field trials is the availability of an efficacious bait well accepted by the target species under field conditions.

For every delivery system tested, the procedure proposed should give basic information on the attainable coverage, technical feasibility, costs and safety aspects of special concern to human beings. All field trials should be carried out by the use of placebo baits without vaccine. The vaccine should be replaced by a systemic marker (e.g. SDM, iophenoxic acid, etc.) and a topical marker (e.g. Rhodamine B, Methylene Blue, etc.).
5. PRINCIPLES FOR THE VOLUNTARY RELEASE INTO THE ENVIRONMENT OF LIVE RABIES VACCINES FOR ORAL VACCINATION OF DOGS

These principles aim at providing help to governments of countries where field trials are being considered, in developing their own regulatory infrastructure and establishing standards for the safe development, use and release into the environment of live rabies vaccines for oral vaccination of dogs.

Governments should consider establishing appropriate regulatory and scientific mechanisms to ensure product(s) safety. Designated national authorities should be responsible for making legislation and ensuring that products and conditions for their use conform with national legislation (and international requirements). These authorities advised by an independent national scientific committee composed of national and if necessary, international experts, are responsible for authorizing the introduction and use of product(s). Producers should make known the characteristics of the product(s) and carry out the necessary experiments to satisfy minimum requirements established at national and international levels. Research workers and project designers should assess the product(s), identify potential hazards and evaluate related risks associated with the introduction into the environment.

International organizations especially WHO (including its network of collaborating laboratories) has a major role to play in providing governments with (a) an assessment of the risks associated with the use and application in the field of each type of product (i.e. modified live vaccine and recombinant vaccine), (b) minimum efficacy and safety requirements for each type of product, and (c) criteria for its distribution in the field. WHO should also inform governments and the scientific community of any new developments.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Bait and bait delivery systems testing

6.1.1 Standardized test for determining dog bait preferences

A standardized test method for determining dog bait preferences and efficacious vaccine delivery to the oral cavity of dogs should be developed and used, where possible, to provide a common basis of comparison between different field studies and investigators. The method should include a detailed sequence of testing schemes (confined, household and free-ranging dogs) manner and duration of bait presentation, a control or reference bait, and minimum sample sizes needed for statistical analyses of data. The specific types of data to be collected should include a description of bait composition, size and origin, the fate of baits, vaccine containers and container contents and the use of a standard field data form(s) and appropriate statistical tests.

Bait candidates should be tested on at least two different sub-populations before being used on a large scale for oral immunization of dogs. These target populations are:

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owned dogs living in households within the area (or country) where oral vaccination is to be applied.

- ownerless and free-roaming owned dogs.

Preliminary studies in Tunisia showed that chicken baits were well accepted by both target populations in different geographical and socio-economical settings. Therefore, chicken head baits may be used as a reference for any new bait candidate.

When the WfM (Wildlife Immunization Model) is considered it may be useful to test any bait candidate in a field trial first by means of the tracking-station method, before further use either for the evaluation of bait delivery systems or for oral mass vaccination in the field on a large scale.

It is proposed that at least two types of baits should be available and used for oral vaccination of dogs. One should be reserved for the distribution of baits to dog owners according to the distribution systems already mentioned.

This type of bait should be clean, easy to handle, and should fulfi the requirements made for foodstuff so that dog owners would not find them objectionable to handle. It may be impracticable to distribute chicken baits to dog owners. Existing artificial baits, however, for instance the polymer bait, are good candidates for this purpose. This bait was accepted by 80% of owned dogs when tested in Tunisia.

A second type of bait could be used for distribution in the field (according to WIM or the hand-out model). For this purpose, chicken baits or the Köfte bait may be useful. The use of artificial baits for this purpose should not be excluded. However, their design should respect the dog's food intake behaviour and food preferences.

6.1.2 Evaluating bait delivery systems

The Consultation was informed that studies (using placebo baits) comparing the various bait delivery systems will be carried out according to WHO document WHO/Rab.Res./93.402, in the near future in Tunisia. The Consultation recommends that similar studies be carried out in as many different socioeconomical and geographical settings as possible. In each situation the utility of various bait distribution strategies (with placebo, no vaccine) should be evaluated according to the principles set out in the WHO document WHO/Rab.Res./93.40 to determine the most effective method appropriate for the described dog ecology parameters.

However, data already available suggest that as an adjunct to parenteral vaccination, priority should be given to bait distribution to dog owners or by door-to-door distribution first and then to dog owners at mobile points. These techniques are expected to significantly increase the overall vaccination coverage.

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2 Suggestions for the development of a research project for the field evaluation of several vaccine bait delivery techniques to vaccinate dogs orally against rabies, by H. Matter.

The increase of the vaccination coverage should be directly proportional to the number of owned dogs which cannot be caught and adequately restrained by their owner for parenteral vaccination (e.g. from 10 to 40% of total population). In most countries the wildlife immunization technique is not expected to reach a much greater number of dogs than the number which can be reached using a combination of parenteral vaccination and oral vaccination by feeding baits to dogs.

6.2 Inactivated vaccines

For inactivated oral vaccines, further research is required to determine alternative antigen delivery systems (e.g. microencapsulated antigens) which provoke sustained and efficacious systemic immune responses when delivered by baits (see Section 2.4).

6.3 Safety requirements for rabies vaccine candidates for oral vaccination of dogs

Dogs are very closely associated with humans, especially with children, in a majority of cultures. The likelihood of direct exposure and of passive vaccine virus transfer to humans is considerably higher for oral dog vaccination than for wildlife immunization programmes.

The group reviewed prior consultation reports as well as the 8th Report of the WHO Expert Committee on Rabies. The following recommendations supersede the relevant sections of these documents.

6.3.1 Modified live vaccines

(a) laboratory tests:

- Considering that dogs under three months of age may form an important part of dog populations in developing countries, and the high probability of contact between young children and puppies, it is recommended that vaccines chosen for oral vaccination should not produce disease in such young dogs when administered per os, at 10 times the field dose.

- The possibility of excretion of vaccine virus in the saliva of the animals described above should also be examined. Following immunization, swabs should be taken daily. No virus should be present after 3-4 days. Any virus recovered should be characterized using monoclonal antibodies or other appropriate procedures.

- In addition, where feasible, at least 10 and if possible 50, of each of the most common local rodent species should be given the field dose of vaccine (i.e. the dose which is contained in a bait) orally and intramuscularly. This may require the use of different virus concentrations and volumes for different species, depending on their weight and size. If the animals so vaccinated fall sick or die from rabies, the use of the vaccine should be reconsidered.

- Relevant local wild or domestic animal species that may take baits should also be given a dose of vaccine orally equivalent to 10 times the field concentration in a volume adapted to body weight.
Any rabies virus isolated from animals in vaccination areas should be examined (e.g. by use of monoclonal antibodies, PCR or other appropriate tests) to ensure that no vaccine-induced rabies has occurred.

(b) monitoring human exposure and providing care

An intense surveillance system should be established to detect any possible human exposure to vaccine. Humans who accidentally come into contact with the vaccine (by mouth, nose, eye or wound) should receive rabies post-exposure prophylaxis. Similarly, persons working with the vaccine and at risk of exposure to it should receive pre-exposure immunization.

6.3.2 Recombinant live virus vaccines (RLV)

(a) laboratory tests

This section deals with the intrinsic safety aspects, i.e. the innocuity of a RLV preparation for the vaccinee. A first evaluation of residual virulence of the candidate strain should be performed by standard laboratory methods, e.g. oral and parenteral inoculation of laboratory animals. Subsequently, oral vaccination of the target species should be performed (e.g. fox, raccoon, dog). The same general guidelines should be followed as have been indicated for Modified Live Vaccines (see previous section). Appropriate laboratory tests (e.g. pock markers, epitopes recognizable by monoclonal antibodies, genetic probes, etc.) can be useful for periodic monitoring of virulence, once its genetic basis has been defined.

Innocuity can be expected from vaccine strains where either genomic deletions or insertional mutagenesis has led to the inactivation of virulence-relevant gene(s). It has been shown, for instance, that inactivation of the thymidine kinase gene leads to vaccinia virus mutants of reduced virulence; similar approaches should be followed for other candidate vector viruses.

Here, other safety aspects are considered, i.e. the safety of a vaccine for non-target species and for humans. Whenever possible, the risks of vaccine virus transmission to humans should be evaluated (e.g. via an immunosuppressed non-target species or a dog re-excreting the vaccine strain).

Real and hypothetical risks must be differentiated. The real risk for non-target species can be definitely established by safety testing of non-target species in the laboratory. Aspects of pathogenicity of the candidate vaccine strain can and should be studied in appropriate laboratory animals, including immunosuppressed animal models, the major non-target species, the most relevant wild vertebrates and in non-human primates, if possible. Thus the course of infection by the RLV must be known, such as its spread from the site of entry, excretion, transmission, contagiousness, and virus persistence. Where approved human vaccines against the carrier virus exist, their use should be considered for those persons involved in vaccine production or distribution.
(b) monitoring human exposure

In the case of vaccinia vectored rabies glycoprotein vaccine (as now developed) where the rabies glycoprotein gene is inserted at the thymidine kinase position in the vaccinia DNA, this vaccine may be considered non-infectious for rabies. Pre-exposure or post-exposure rabies vaccination is **not** recommended for persons exposed to this vaccine. However, persons exposed to the vaccinia recombinant rabies virus should be followed-up. Paired sera should be obtained following the exposure and afterwards (e.g. after 30 days). Treatment should be symptomatic if illness occurs. Appropriate samples should be obtained for virus confirmation if lesions develop. WHO collaborating centres should be contacted for assistance. Risks from other recombinant vaccines must be evaluated on an individual basis as and when such vaccines are developed.

A hypothetical risk is the recombination of the RLV vaccine viral genome with that of another virus, with the resulting recombinant possibly possessing higher virulence and greater epidemiological potential. The realization of this hazard has not been borne out for poxviruses either in the laboratory or in nature. Nevertheless, the possibility of other RLVs to recombine, to cause persistent infections, or to become oncogenic should be kept in mind and investigated. This applies especially to other potential vector viruses whose DNA replication is in the nucleus.

6.3.3 General considerations on risk

In summary, a safe vaccine virus candidate should:

- not acquire virulence during replication in the vaccinee;
- not be oncogenic in the vaccinee;
- fulfil the requirements for target and non-target species established in this document (see Sections 6.3.1 and 6.3.2);
- not recombine with viruses occurring in nature to result in viable pathogenic progeny;
- demonstrate that its possible excretion is not hazardous;
- be evaluated for potential public health risks associated with its use;
- bear at least one genetic marker for identification.

6.3.4 Special safety considerations

The use of live vaccines should be discouraged when the risk of unintentional exposure to severely immunocompromised populations is deemed high, because of the chance of enhanced viral replication, altered tropism or untoward adverse events. Conversely, inactivated vaccines do not appear to represent a danger to immunocompromised individuals. Research on inactivated oral rabies immunogens for dogs is in progress. Some encouraging results have been obtained in raccoons. However, future results will require careful assessment before the concept of an inactivated rabies oral immunogen for dogs is abandoned as the next obvious generation of vaccine. In lieu of an alternative, several candidate attenuated or recombinant rabies viruses appear to offer safe, efficacious and economical solutions for the oral immunization of immunocompetent hosts.
The candidate live vaccine should also be tested in primates, such as chimpanzees, baboons, rhesus monkeys, etc. At least 10 animals of one species should be given 1 ml or more of 10 times the intended field dose of vaccine by direct instillation into the oral cavity. Whenever sufficient numbers of animals are available, the vaccine should also be tested in a similar number of immunocompromised primates. No vaccine-related mortality should occur during an observation period of at least 90 days. Tests for rabies and vector virus antibody should be made before inoculation of the vaccine and at the end of the experiment.

Considering that incubation periods of rabies following MLV inoculation in primates may be in excess of 90 days it is suggested, when appropriate, that primates be administered a dose of modern, potent, inactivated rabies vaccine and serologically evaluated for an anamnestic response or possible manifestation of the "early death" phenomenon.

Candidate vaccines should also be given by oral, intracerebral, intramuscular and other relevant routes to nude and SCID-mice or other immunodeficient laboratory animal models. Results should be compared with those observed in immunocompetent laboratory animals.

Levels of vaccine virus excretion during the days after oral vaccination should be determined. An appropriate quantitative test should be established for that purpose. Subsequent experiments should involve appropriate immuno-suppressed models to determine the effects of these virus titres.

6.4 Additional precautions to be taken before initiating field trials with vaccine-loaded baits

Studies using baits containing a topical marker instead of vaccine should be conducted prior to releasing any live vaccine to assess the magnitude/frequency/nature of contacts between dog owners/vaccine/dogs.

All partners involved in field trials for the oral vaccination of dogs (i.e. producers, research workers, research designers, national designated authorities) should adhere to the principles set out in Section 6.

WHO staff and/or staff from relevant WHO collaborating centres should be closely associated with designated national authorities or independent national scientific committees when pilot research projects using these products are considered.
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## ANNEX 2. SAG₂: SAFETY TESTS IN NON-TARGET SPECIES

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Dose</th>
<th>Duration of observation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic cat</td>
<td>5</td>
<td>oral</td>
<td>10⁹ PFU</td>
</tr>
<tr>
<td>European rodents:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Apodemus flavicolis</em> or</td>
<td>15</td>
<td>oral</td>
<td>10⁷.7</td>
</tr>
<tr>
<td><em>A. sylvaticus</em></td>
<td>15</td>
<td>musc.</td>
<td>10⁷.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>cerebr.</td>
<td>10⁷.5</td>
</tr>
<tr>
<td><em>Microtus arvalis</em></td>
<td>9</td>
<td>oral</td>
<td>10⁷.7</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>musc.</td>
<td>10⁷.5</td>
</tr>
<tr>
<td><em>Clethrionomys glareolus</em></td>
<td>11</td>
<td>oral</td>
<td>10⁷.7</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>musc.</td>
<td>10⁷.5</td>
</tr>
<tr>
<td><em>Arvicola terrestris</em></td>
<td>2</td>
<td>oral</td>
<td>10⁷.7</td>
</tr>
<tr>
<td><em>Rattus norvegicus</em></td>
<td>15</td>
<td>oral</td>
<td>10⁷.4</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>musc.</td>
<td>10⁷.4</td>
</tr>
<tr>
<td>European wild birds:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Corvus frugilegus</em></td>
<td>9</td>
<td>oral</td>
<td>10⁸.5</td>
</tr>
<tr>
<td><em>Corvus corone</em></td>
<td>7</td>
<td>oral</td>
<td>10⁸.5</td>
</tr>
<tr>
<td>African species:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civet (<em>Civettictis civetta</em>)</td>
<td>6</td>
<td>oral</td>
<td>10⁹</td>
</tr>
<tr>
<td>Baboon (<em>Papio ursinus</em>)</td>
<td>5</td>
<td>oral</td>
<td>10⁹</td>
</tr>
</tbody>
</table>

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