



2nd WHO CONSULTATION ON ORAL IMMUNIZATION
OF DOGS AGAINST RABIES

Geneva, 6 July 1990

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1. INTRODUCTION

The first WHO Consultation on Oral Immunization of Dogs was held in Geneva from 26-27 February 1988 (report - WHO/Rab.Res./88.26). On the basis of the recommendations of the first consultation together with those of the WHO Consultation on Requirements and Criteria for Field Trials on Oral Rabies Vaccination of Dogs and Wild Carnivores, Geneva, 1-2 March 1989 (report WHO/Rab.Res./89.32), a number of candidate vaccine strains, baits and bait delivery systems were tested under laboratory (vaccines) and field (baits and bait delivery) conditions.

The objectives of the second consultation were to: (a) review the results of research conducted since the first consultation; (b) propose standardized protocols for the implementation of further experiments on vaccines, baits and bait distribution; and (c) identify future activities.

Dr G. Baer was elected Chairman and Dr D. Fishbein, Rapporteur. The list of participants is given in Annex 1.

2. REVIEW OF RESEARCH CONDUCTED ON ORAL IMMUNIZATION

2.1 Candidate Vaccines

2.1.1 Vaccinia-rabies recombinant (VRG), SAG1 and ERA strains

Two separate experiments were carried out on adult beagles bred in colonies at two French laboratories.

a) Trials with the vaccinia rabies recombinant virus

The vaccine obtained from Rhône Mérieux laboratory consisted of genetically modified vaccinia virus (Copenhagen thermosensitive 26 strain) expressing the foreign glycoprotein G of the rabies ERA strain. Two doses of the vaccine were administered by direct deposit into the mouths in two groups of 4 dogs (titre of virus administered: $10^{9.6}$ and $10^{8.6}$ PFU respectively). Five of the vaccinated dogs were kept in contact with five naïve dogs (in pairs) and five unvaccinated dogs were kept separately as control.

All 18 dogs were challenged 69 days later, with $10^{5.5}$ MICLD₅₀. Fourteen succumbed to rabies, and the four which resisted belonged to the group vaccinated with $10^{9.6}$ PFU. These four dogs exhibited antibody titres ranging from 3.6 to 13.3 IU/ml, 28 days after vaccination.

b) Trials with the SAG1 and ERA virus

The SAG1 vaccine obtained from Dr A. Flamand, CNRS, Paris, is a double mutant of the SAD Bern strain.

The ERA strain was isolated from commercially available vaccine plaque purified at the Pasteur Institute in Algiers and received in Nancy from Dr Ben Mansour. One

dose of SAG1 vaccine ($10^{9.8}$ PFU = $10^{7.7}$ MIC LD₅₀) and one dose of ERA vaccine ($10^{9.3}$ PFU = $10^{6.8}$ MIC LD₅₀) were given to two groups of 6 dogs, in a capsule hidden in a chicken head.

Three weeks later, the 12 dogs and 6 unvaccinated (control) dogs were challenged with 10^4 MICLD₅₀ of a street canine strain (previously titrated in 12 dogs).

Five out of 6 control dogs died as well as 4 out of 6 in the ERA group and 1 out of 6 in the SAG1 group. In this latter group the antibody titre ranged from 0.04 to 0.15 IU.

Oral vaccination of dogs seems feasible by distribution of baits containing at least $10^{9.8}$ PFU of modified live virus vaccine per dose.

2.1.2 SAD-Bern & SAG1

In order to evaluate the possibility of orally vaccinating dogs against rabies, two ERA-derived strains were tested in Tunisia under laboratory conditions: SAD-Bern (experiment 1) and SAG1 (experiment 2).

In the two experiments, the dogs were divided into three groups:

Group A: (7 dogs) received $10^{7.5}$ TCID/ml of SAD-Bern or SAG1 by direct oral deposit of 1 ml of vaccinal suspension.

Group B: (7 dogs) received a tenfold dilution of the same vaccines ($10^{6.5}$ TCID/ml).

Group C: two control groups consisting of 4 dogs for experiment 1 and 7 dogs for experiment 2.

SAD-Bern virus excretion was observed in the saliva of one dog in group A on Day 3, but isolation was not confirmed by the WHO Collaborating Centre for Reference and Research on Viral Zoonoses, Bern.

No salivary excretion was detected in the case of SAG1 (tested on Days 1, 3, 5 and 10).

To date, efficacy results concern only SAD-Bern (experiment 1) as experiment 2 is still ongoing. They are the following:

Good serological response at Day 21, 45 and 120 (measured by RFFIT in the Institute of Microbiology, Bern) in group A and group B (with a significantly higher response in group A);

After the challenge performed on Day 120 with a Moroccan street virus (provided by Dr J. Blancou, LERPAS, Nancy), all dogs survived in groups A and B and all the 4 control dogs in group C died of rabies.

2.1.3 Human adenovirus 5 rabies recombinant (HAD5RG) in carnivores

This vaccine was tested orally in wildlife animal species, mainly skunks (145) but also foxes (37) and raccoons. A vaccine titre above 10^8 usually immunized skunks and foxes after direct oral instillation. Higher titres were required if the vaccine was included in the bait (blister pack, sponge and Dupont baits were tested). Animals showing seroconversion resisted challenge.

Six raccoons received 1 ml of vaccine (titre 10^8) in a fishmeal polymer bait. All seroconverted and were protected. Only approximately 50% seroconverted and were protected by deposition of 1 ml of vaccine with a titre of 10^7 .

Preliminary testing was conducted in dogs. Two dogs were inoculated by subcutaneous route and two more by intranasal instillation of 5×10^5 PFU of HAd5-RG. The four animals seroconverted. No challenge was carried out.

2.1.4 Inactivated vaccines for oral immunization

Over the last three years, studies with inactivated rabies virus vaccines for oral vaccination of raccoons (approximately 1.0 ml by oral deposition, with rabies challenge three to four months after vaccination) have suggested that:

- large concentrations of whole virus vaccine ($\geq 800 \mu\text{g}$) are necessary for protection;
- vaccine efficacy may be dependent upon virus strain (ERA vs PM);
- the anamnestic response differs between booster doses of oral live versus inactivated vaccine;
- baculovirus vaccines may be useful to produce large concentrations of rabies glycoprotein and can protect raccoons;
- these preliminary results and their potential implications for dog oral immunization must be viewed with cautious optimism until corroborated by other WHO laboratories with a variety of species and protocols.

2.2 Baits and bait delivery systems

2.2.1 Testing of four bait types in Tunisia

Four different baits were simultaneously tested in order to evaluate their acceptability by dogs. Characteristics of the baits and protocols are summarized below:

Types of Bait	Merguez (MZ)	Fishmeal Polymer (MX)	Chicken head (CH)	Sponge (SP)
Comments	Tunisian sausage with donkey meat and rice	Manufactured bait	Chicken head	Attractant in a plastic sack
Vaccine container	Straw	Plastic capsule	Bern capsule	Sponge
Number of dogs receiving bait	50	50	50	50

In all cases vaccine was replaced by a dye (Rhodamine B or methylene blue) in order to facilitate visualization of the contact of the content of the vaccine pack with oral mucosa.

Baits were always distributed in the same order: MZ, MX, CH, SP as owned-dogs were encountered. Only one bait was given to each dog. Each bait type was given to 50 dogs. The results were as follows:

Bait	Acceptability (A)	Positive contact with dye (P)	Vaccinal Success (A) x (P)
MZ	56%	46.4%	26%
MX	80%	77.5%	62%
CH	96%	98.0%	94%
SP	66%	30.3%	20%

Chicken heads were significantly better accepted and led to the highest level of positive contact with the dye. In addition, chicken heads have the advantage of being very cheap and unattractive for man (and children) in Tunisia.

Fishmeal polymer came in second position with a vaccinal success of 62%. MX could, however, be interesting should a manufactured bait be required.

2.2.2 Dog bait preference field tests in Mexico

Three different bait evaluation techniques were assessed according to the following tests:

Test 1. Each dog encountered in the street was given one bait, the bait type

given (A-D) was determined by a randomized listing provided to each team. One team member offered the bait and timed events, while the second recorded information on a field data sheet.

Test II. This test was conducted in the same manner as Test I, except that only dogs physically located within households were offered baits. As in Test I, the same baits were offered, one per dog, in random order.

Test III. This test, using only household dogs, was a two-choice bait preference test. The test was conducted by simultaneously placing two baits on the ground in front of the dog and recording which was taken first.

Highlights for the above three tests are as follows:

Test I

Mean chewing times did not differ greatly between the 4 bait types used. The characteristics of the baits were:

Bait A - A medium length dog biscuit having two drilled holes lined with paraffin wax and containing 1.0 ml of dye water (devised by Centers for Disease Control, Atlanta, Georgia);

Bait B - A standard length (1.5 x 5.5 cm) foam sleeve hardened corn bait containing a paraffin wax ampoule that held 1.0 ml of dye water (devised by Denver Wildlife Research Center - DWRC, Denver, Colorado).

Bait C - Same as bait B above, but approximately half the length (1.5 x 3.0 cm - devised by DWRC)

Bait D - A length of beef "hot dog" cored to accept a paraffin wax ampoule and air dried and hardened for 4 days at ambient temperature resulting in a bait 1.5 cm x 4.5 cm in size (devised by DWRC).

The percentage of times pieces of bait fell to the ground while the bait was being eaten did not vary greatly according to bait type. On an average pieces of bait fell to the ground 76 per cent of the time.

Regardless of bait type, all baits were completely consumed 80.5% of the time (range 70.8-84.6%); partial consumption was less than 1% and about 15% of baits were untouched.

Liquid-filled, intact ampoules were found discarded on the ground following partial or complete bait consumption about 8% of the time (5/62), regardless of bait type.

Broken ampoules (usually pieces or chewed portions thereof) were found on the ground approximately 40% (25/62) of the time, regardless of bait type (except bait A which had no ampoule).

The mean area of spilled dye water (simulating vaccine) found on the ground and the percentage of baits with leaking ampoules varied considerably by bait type, although small sample sizes make any comparisons tenuous.

Test II

Mean chewing times varied considerably; the mean time for bait A was about 2-3 times that for the other three baits. However, this may have been simply a function of size; bait A was larger than the other three types.

Pieces of bait falling to the ground during mastication was observed 77% of the time and did not vary greatly between bait types.

Baits were completely eaten 89% of the time (range 84-96%), partial consumption was infrequent (4%), and uneaten baits were recorded for 7% of total trials.

Intact ampoules and pieces of ampoules or chewed portions thereof were recorded at 3% and 14%, respectively.

For baits B, C, and D, the mean ground area covered by spilled dye water was 2.0, 5.6 and 8.4, respectively, while percentage spillage was observed in 32%, 25%, and 70% of the time, respectively for baits B, C and D.

Test III

All data from this two-choice bait test have not yet been tabulated.

On average, baits were completely consumed 78.5% of the time (range 71.1-84.5%). Partial bait consumption and the frequency of baits not consumed was 9.7% and 10.9% respectively.

Statistical analyses of the complete data are required to rectify these preliminary results. Dogs selected baits A, B, and C 43-50% more often than D; bait C was selected 37% more often than B, A was selected 31% more often than C, whereas A and B baits were equally accepted.

2.3 Comparison of parenteral versus oral vaccination strategies

2.3.1 In rural Mexico

Human rabies in Puebla primarily occurs in residents of small towns. In three such towns, dog populations and different vaccination strategies were studied: extensively publicized central point (town A), less publicized central point (town B), and door to door (town C). Dog population size and vaccination coverages in A, B & C are given below:

	Town A	Town B	Town C
Dogs:Humans	696/1 810	314/1 227	562/2 222
Ratio	1:2.6	1:3.9	1:4.0
Vaccinated	71%	82%	86%

Following this, the acceptability of five canine baits in Town A was studied. Commercial dog biscuits were the best accepted bait (88% of dogs), followed by fish flavoured sponge (69%), corn-flavoured sponge (67%), fish-flavoured commercial polymer (50%) and commercial wax bait (10%). Control of canine rabies in rural areas will be more efficient with an oral vaccine, but will still be extremely labour-intensive.

2.3.2 In Tunisia (suburban area)

Between October 1989 and February 1990, the accessibility of dogs to parenteral vaccination was compared with oral vaccination in a suburban area of Tunisia.

Baits consisted of chicken heads incorporating plastic capsules filled with a placebo liquid and Rhodamine B as a biomarker.

Of owned dogs 77.5% ate baits and showed evidence of superficial staining of the mouth cavity by Rhodamine B, whereas successful parenteral vaccination was achieved in 91% of these dogs. Overall, 98.0% of owned dogs were accessible by parenteral and oral vaccination.

An optimal combination of the two vaccination methods has been established. It consists of an attempt to administer vaccine by the parenteral route for 120 seconds, and, if unsuccessful, shift to a vaccinal bait for 90 seconds. If oral vaccination is also unsuccessful, revert to the first technique and intensify efforts to restrain the dog for vaccine injection.

In addition to owned dogs, ten out of 18 unowned dogs living within the study area were "vaccinated" with the placebo vaccine bait. Baits were presented directly or placed in proximity of dog groups. By using the two techniques, the global "vaccination" rate reached 75.5% of the total population.

2.4 Other studies related to oral immunization of dogs

2.4.1 Estimating dog population size in an urban area

In 1990, a study on the biology of the dog population in an urban area of high human population density was carried out in Tunisia. Estimation of the total dog population size was based on a Capture-Mark-Reobservation technique using the Peterson/Bailey formula (see Guidelines for dog rabies control, VPH/83.43, Rev.1, page 2.4-2.6). One of the assumptions for using this formula is that each dog in the population has an equal probability of reobservation. As this assumption is not

fulfilled in most cases, reobservation conditions can considerably affect results obtained by the above technique. This was thought to be especially true in urban areas where the probability of reobservation of owned dogs is highly related to the level of confinement imposed by the owners. In most instances tied up or enclosed dogs are unobservable from the street.

All owned dogs in the study area had been marked by collars of different colours according to whether they were free-roaming (colour "F") or tied up or enclosed (colour "E") at time of capture and marking. During the reobservation passages it was thus possible to recognize the status of every dog's degree of confinement at time of marking. This allows an estimation of possible biases in population size estimates calculated with the Peterson/Bailey formula, by selecting specific groups of dogs in the reobserved samples. For example, differences in estimations of dog population size were as high as 80% when beside unowned (unmarked) dogs only dogs wearing the colour "E" collar or only dogs wearing the colour "F" collar were taken into consideration in the reobserved samples.

As a consequence, the calculated number of unowned dogs (by using again the Peterson/Bailey formula) was also subjected to important overestimations. In the study area under the above described extreme but theoretically possible reobservation condition (only dogs with "E" collars in the sample), the calculated number of unowned dogs was as many as four times greater than what was known to be the best estimation of the real number of unowned dogs.

A further amelioration of population size estimates became possible when the total number of dogs wearing colour "F" collars which were actually free-roaming at time of reobservation ($F_{(t)}$) was first estimated and then integrated into the Peterson/Bailey formula:

$$F_{(t)} = F_o \left(1 - \frac{f_e * v_f}{f_f * v_e + f_e * v_f} \right)$$

$$N = \frac{F_{(t)} (f_f + n + 1)}{f_f + 1} - F_{(t)}$$

$F_{(t)}$: Estimation of the total number of dogs wearing colour "F" collars which are free-roaming at time of reobservation (t).

N: Estimation of the total number of unowned (unmarked) dogs.

F_o : Total number of observed dogs wearing colour "F" collars (at time of marking).

f_e : Number of observed dogs wearing colour "F" collars which are tied up or enclosed at time of reobservation (t).

f_f : Number of observed dogs wearing colour "F" collars which are free-roaming at time of reobservation (t).

n: Number of observed dogs wearing no collars at time of reobservation (t).

v_f : Mean visibility of free-roaming owned dogs (number of owned dogs free-roaming and visible from the street at time of marking divided by the total number of free-roaming owned dogs at time of marking).

v_e : Mean visibility of owned dogs tied up or enclosed (number of owned dogs tied up or enclosed and visible from the street at time of marking divided by the total number of tied up or enclosed owned dogs at time of marking).

It was concluded that, especially in urban areas, different marks should be used to identify owned dogs according to their level of confinement to which probability of reobservation is highly related. This practice will permit excluding from reobservation all dogs wearing the "E" mark, reobservation probability of which is very low.

Reobservation should be limited to the free-roaming part of the owned dog population (dogs with the "F" mark, still free-roaming at time of reobservation) and to the unowned (unmarked) dogs. The probabilities of reobservation of these two latter groups are more homogeneous and therefore more in line with the assumption of equal reobservation probabilities required for the use of the Peterson/Bailey formula.

2.4.2 Parenteral vaccination campaign in Nepal

It was planned to vaccinate a minimum of 75% of the dog population in Lalitpur against rabies within one month. Vaccination teams stayed in a given place for several hours and vaccinated any dogs presented. The teams were usually located near cold stores, market places, temples or other well-frequented spots throughout the city. All dogs were vaccinated, registered, and marked with a plastic collar.

To estimate the percentage of vaccinated dogs, the areas where vaccination teams had recently operated were regularly toured. In most instances, control tours immediately followed the campaign activity, so that collar losses could be considered minimal. A daily collar loss rate of 0.75% was, however, taken into consideration and the percentage of collared dogs was adjusted accordingly for individual controls as well as for the total.

From direct observation the overall vaccination coverage after the regular campaign was estimated to be 66.3%. With correction for collar losses, the coverage was calculated 67.6%. Vaccination coverage could also be estimated from the relation of the number of vaccinated dogs to the total dog population. Two estimates of the total dog population were made, applying Bailey's direct sampling method and using the vaccination and reobservation data. Dogs that were vaccinated in the follow-up campaign and at the Veterinary Hospital increased the total of dogs vaccinated during the first round. With the dog population estimate based on mark-recapture data, vaccination coverage was about 80%.

2.4.3 General considerations on feasibility of oral vaccination projects

The oral vaccination projects that have been carried out have shown that people, especially children, take a great interest in any non-routine activities. An oral or parenteral vaccination campaign will, therefore, draw much attention from the public.

When contemplating the initiation of field trials with vaccine bait, attention should be paid to the following:

The customs of the human population have to be taken into account when planning for an oral vaccination campaign. The interference of man with baits designed for

dogs must be minimal. Hand-feeding of baits or specific placement with recovery of baits will be the only techniques that can prevent the public or other non-target species from coming into contact with potentially hazardous modified live or recombinant live vaccines.

In an oral vaccination campaign dog owners cannot be asked to bring their dogs to a specific point for vaccination. This method can already cause problems in a conventional campaign (aggressive interactions between dogs, etc.) and will do so further when food (bait) is involved.

In addition, dogs may refuse to take baits when they are not within their accustomed territory. Whether baits should be handed to dog owners in order to be fed to dogs at home is mainly a question of the risks associated with the vaccine/bait system chosen.

When hand-feeding and specific placement with recovery is applied, it is questionable whether the unowned dogs that escape a parenteral campaign can be reached. These animals are not easily accessible, and if so, they may refuse to eat bait in the presence of man.

With an oral vaccination campaign most of the non-vaccination activities like information, transport, etc., would most likely be equally time-consuming. Depending on the baiting system chosen additional time is needed for bait preparation. The baiting technique itself will probably exert the largest influence on the overall time budget of the campaign. With hand-feeding of baits and/or specific placement and recovery of baits the time-efficiency of oral vaccination may not be higher than with parenteral vaccination at neighbourhood centres.

With the presently available modified live (ML) recombinant live (RL) vaccines, oral vaccination campaigns in densely populated urban areas of South-East Asia may not appear to be superior to conventional (parenteral) vaccination campaigns. The scarcity of potent vaccines for the treatment of humans who accidentally come into contact with ML vaccines for dogs, the relatively low hygienic standards, and the risk for humans associated with ML and potential risks associated with certain carriers for RL vaccines necessitate distribution methods (hand-feeding of bait, specific placement with recovery of bait) which minimize the probability of hazardous contacts.

3. GUIDELINES FOR FURTHER RESEARCH INTO ORAL IMMUNIZATION OF DOGS

3.1 Determining oral vaccine efficacy in the laboratory

3.1.1 Administration of vaccine

The volume to be administered is 1.0 ml in a 1.0 ml syringe.

Test animals should not be sedated; vaccine must be placed drop by drop in the centre of the tongue using a needle-less syringe; there should be no contact with tissues.

3.1.2 Vaccine titration

For Modified Live Vaccine (MLV), infective titres can be measured in mouse (MICDL₅₀/ml) or in tissue culture inoculation (e.g. TCID₅₀/ml or PFU/ml). The first dilution of the vaccine used should be that obtained at the production level. If found effective, several serial dilutions (e.g., tenfold, etc.) should be tested. The titre (per 1.0 ml) must be stated (see above). The diluent should be cell culture medium (e.g. PBS or Hanks) without serum. Vaccine titre should be determined immediately after vaccination.

3.1.3 Dog characteristics

Test dogs should only be used once per rabies vaccine experiment. There should be at least five dogs in each experimental group and a minimum of five dogs in the control group. Dogs should be of known origin, of standard size and breed, and stratified to group by sex and reproductive conditions. They should be at least 6-12 months of age and of known age where possible. They should be free of known diseases and should have no history of anti-rabies vaccination. They should be vaccinated against common canine diseases (e.g., distemper, hepatitis, etc.), If anti-helminthics are administered they should be given no less than one week before experimentation. The dogs need to be individually identified. They should be separately housed immediately after vaccination, if possible. Routine veterinary care and husbandry should be performed according to humane conditions.

3.1.4 Immune response

Anti-rabies serology (either in vitro or mouse inoculation test) should be conducted before vaccination; dogs should have no deducible rabies virus neutralizing antibody. It is suggested that the post vaccination serology begin on Day 7, be obtained weekly thereafter through Day 28, and on the day of challenge. Aliquots of serum should be stored frozen at -20°C or lower; repeated freezing and thawing of specimens should be avoided. Serum titre should be expressed in IU/ml (compared to the international reference serum).

3.1.5 Virus challenge

It should preferably be a well-characterized street virus of dog origin (salivary gland material). If the LD₅₀ data for dogs is not known for the challenge strain, it should be determined by the usual techniques. A concentration sufficient to kill 80% of controls should be administered. The inoculation route and site(s) of inoculation at time of challenge should be the same as those used to determine the LD₅₀ (e.g. temporal muscle). In preliminary studies dogs should be challenged 1-3 months after vaccination using a 20-21 gauge needle. The inoculum should be titrated in mice following the challenge of dogs. Dogs need to be observed daily and euthanized at the first definitive clinical signs of rabies (e.g. self-mutilation, recumbent paralysis, or inability to drink or feed over a 48-hour period). The diagnosis of rabies must be confirmed in dogs that die or are euthanized. Trials with field dogs should follow the protocols recommended for laboratory dogs as closely as possible.

3.1.6 Vaccination of dogs by baits

The vaccine container and bait should be presented together. Food should be withheld for 24 hours. The bait should be given free-choice (i.e., no pressure is imposed on the dog to take it). Baits should be observed as frequently as possible and removed after 24 hours. Conditions of the bait/container need to be examined and recorded to determine the level of contact and consumption.

The vaccine presentation should optimize the release of the vaccine to the target tissues. The response of the dog should be observed with regard to the way the vaccine is included in the bait. Depending on the vaccine and the bait-type, the vaccine may be presented as an unprotected liquid or lyophilized solid, as a liquid in a protective container (e.g., sachet) or as a tablet or microencapsulated particle. The vaccine presentation should be integrated as much as possible with the bait matrix so that the vaccine cannot be separated from the matrix and rejected. Such rejection may be via rejection of a bait container or rejection of large particles of the bait-containing tablets, capsules etc. Following the establishment of basic efficacy, innocuity testing should be determined as reported in document WHO/Rab.Res./89.32 in Section 2.2.2 Safety requirements of modified live vaccines:

"A. Innocuity testing

Dogs

(a) The vaccine chosen should not produce any disease in 10 young dogs (3-6 months old) when administered orally at a 10-fold concentration of the quantity recommended for field use.

(b) To trace the possible excretion of vaccine virus in saliva of orally vaccinated dogs, 10 dogs administered 10 times the field concentration of vaccine should be swabbed daily for three days and no virus should be present after three days. Any virus recovered should be characterized by monoclonal antibodies.

(c) To test for possible latency, 10 dogs given 10 times the field concentration orally should be sacrificed and brain and salivary gland examined at 6 months. Any vaccine strain leading to latency should be rejected."

3.2 Testing bait composition and bait delivery

3.2.1 Bait preference trials

Preliminary experiments carried out to determine dog bait preference should be conducted using the classical two-choice food preference or other controlled comparisons. Once a bait type has been selected, a one-bait test should be used to determine the up-take rates by the dog population.

Criteria to be considered during the immediate observation phase of the trial include: length of the test period; time until physical contact with the bait; chewing time; dye markers on mouth or teeth; quantity of dye liquid on ground; quantity of vaccine container or matrix remaining on the ground.

Further observations on the container should include: detection of container by dog; state of container at completion of trial (discarded intact, chewed and discarded, swallowed).

Additional factors which need to be considered under conditions where observation is delayed should include: number of bait remains; dye markers on the ground; dye marker on target and non-target species; dye marker in faeces and urine; hiding/burying of bait (location determination by radio transmitters).

3.2.2 Bait types

It should be noted that a number of bait types have already been used in trials on dogs (e.g., chicken head, dog biscuit, corn-flavoured/foam sleeve (CFFS), fishmeal polymer bait cylinder, fishmeal polymer bait regular, sponge bait, Canadian wax/tallow bait, hot dog bait, Merguez sausage bait). As a rule, reference and comparison should be made to one or more of these bait types when conducting new field bait trials.

3.2.3 Observation categories for bait components

The response of dogs to baits during field trials should be observed and noted with regard to the following categories: bait matrix, vaccine container, attractants/repellents and biomarkers.

For each of the above categories, attention should be paid to the following characteristics:

- a) Bait matrix: Physical configuration of bait (size, shape); bait colour; texture (hardness, friability), melting point and physical decomposition under storage and field conditions (e.g. temperature freeze/thaw, rain and moisture, insect damage, bacterial decomposition).
- b) Vaccine container: shape, size, texture, etc.
- c) Odour attractants/repellents: The range of an attractant should be evaluated. If it is long range, then distance from which bait is identified by sight or smell should be determined. For cases of short range attractants, feeding acceptance of the matrix, vaccine container and vaccine should be noted.

It is necessary to determine if particular non-target species eat or reject the baits containing repellents.

- d) Biomarkers: Ideally a bait should contain two biomarkers - one for the bait matrix, one for the vaccine. This is particularly important where the bait is non-homogeneous, i.e., if the bait matrix and vaccine container can be separated

by the dog during ingestion. Biomarkers should be safe for target and non-target species, particularly human beings. They should also be innocuous to the vaccine and not affect the acceptance of the bait by the dog.

Two categories of biomarker should be taken into consideration in bait trials:

- Topical: These are dyes such as Rhodamine B or Methylene blue which can be seen on the animal or on the ground by direct observation.
- Systematic: These are compounds such as Iophenoxic acid or tetracycline, which require phlebotomy, biopsy or post-mortem samples for detection.

When establishing the use of a biomarker in a baiting system, the effective dosage and duration should be measured in the bait and target dog population. The established dose should then be standardized in all studies to maintain uniformity of marker in results.

3.2.4 Bait manufacture

In order to achieve an effective vaccine bait for large-scale usage, the following points should be considered when designing the bait system. The bait should be: acceptable to local dogs; locally producible; as inexpensive as possible; producible in large repeatable batches from year to year; storable in large quantities for short-term packaging and handling to distributors and for long-term frozen storage.

3.2.5 Bait distribution methods

The following methods should be considered when distributing baits to dogs during small scale bait preference experiments and during large scale trials. The trained research team may distribute baits either directly to accessible dogs or to owners of accessible dogs or to local personnel (e.g., neighbourhood representatives). They in turn distribute baits to the dogs or their owners or to a local trained distributor who distributes baits by selective broadcasting either on foot, by bicycle or by motor vehicle.

Studies are needed to determine the bait density needed to reach a high percentage (70%) of dogs. Trial densities should include a range of at least one to five baits per dog until the most efficient level is determined.

3.2.6 Timing of distribution

Foraging times for dogs in the target area should be known. When defining the timing of bait distribution, the following parameters should be taken into consideration: time of day (morning, midday, evening, night, garbage collection time); day of week (garbage pick-up day, market day, festivals, cattle dipping day); season of year and weather conditions; depredation by invertebrates and rodents.

Annual baiting campaigns should be designed with the above considerations in mind. It is important when establishing any new dog vaccination campaign, that the continuity and uniformity be maintained during the initiation stage and in particular from year to year.

3.3 Organizing field trials for oral immunization of dogs

The following steps should be followed:

3.3.1 Select site

An area should be selected where rabies is endemic or epidemic. Preferably a community should be chosen where cooperation can be easily mobilized. Community information and education should be carried out prior to initiating the trial.

3.3.2 Estimate canine population size

A map of all streets and houses in the town should be obtained or made. The existing rabies surveillance system and data should be evaluated. Sample size (small for large community and vice versa) should be calculated.

3.3.3 Estimate size and accessibility of canine population

An estimation of the total dog population size should be made using Capture-Mark-Reobservation techniques. The heterogeneity in the reobservation probabilities of the different groups of owned dogs must be taken into consideration especially in urban or suburban areas. Different marks should be used to identify the different groups of dogs composing the owned dog segment of the population (see 2.4.1). Accessibility to the control measure to be applied (e.g. vaccination by the parenteral route, oral immunization) should be defined. On that basis a count of accessible dogs to the control measure to be applied should be made in the study area. Counting of accessible owned dogs can be made either during or after the capture-mark phase.

3.3.4 Estimate accessibility for oral vaccination

First of all, one of two distribution methods indicated below should be selected:

a) House-to-house distribution

Determine bait acceptance (with or without an actual vaccine) and compare to parenteral vaccination. Baits can be presented after or before parenteral vaccination or randomized with parenteral vaccination so that the effect of the parenteral vaccination on the acceptance of the bait can be estimated.

b) Street or field distribution

Vaccine bait should either be directly presented to dogs on the street or be field distributed (scattered). In order to evaluate their acceptance, it is advisable to use dogs belonging to the accessible and observable category.

3.3.5 Determine efficiency

Efficiency (time required) and cost per vaccinated dog (including vaccine) of oral vaccination and parenteral vaccination strategies should be determined. Efficiency and, therefore cost, may vary from one dog to another. Preliminary studies should calculate separate cost estimates for different groups of dogs. These groups are characterized by the following: a) accessible to parenteral vaccination (at a central point or in a house-to-house campaign), b) accessible to oral vaccination (at a central point or in a house-to-house campaign, c) accessible through street distribution.

3.3.6 Determine optimal strategy

Optimal vaccination strategy (parenteral, oral, or a combined programme) should be determined. The final approach must be inexpensive, simple and effective.

3.3.7 Evaluate programme

An evaluation should be performed soon after the programme is completed.

4. FUTURE ACTIVITIES

4.1 Wistar Institute, Philadelphia, USA

The Wistar Institute anticipates:

- a) promoting laboratory testing of V-RG recombinant vaccine in various baits for basic efficacy in dogs.
- b) investigating the immunogenicity and efficacy of inactivated vaccine in dogs by oral deposition, according to suggested protocols.
- c) continuing placebo bait contact and acceptance by dogs in the field in various socioeconomic and geographic settings in Thailand to select the most appropriate bait type.

4.2 Veterinary Research Laboratory, Harare, Zimbabwe

The VRL plans to resume its work on oral vaccination of dogs. VRL will:

- a) re-apply to the Ministry of Health to use vaccinia (and other recombinant vaccine(s)).

- b) conduct, if permission is granted, safety trials in non-target species (Peraomys, Tatera, mongoose, civets).
- c) carry out further field trials with bait uptake around rural settlements in communal lands using chicken heads and dog biscuits as a reference.
- d) identify areas where oral vaccination of dogs would be necessary due to inadequate coverage with traditional parenteral vaccination campaigns.
- e) undertake, assuming a recombinant vaccine is found to be safe in non-target species, field trials of this vaccine in dogs.

4.3 Animal Diseases Research Institute, Nepean, Canada

ADRI will pursue research on efficacy and safety of HAd5RG in target and non-target species, including dogs.

4.4 Institute for Veterinary Research, Tunis, Tunisia

This Institute will complete the laboratory work on SAG, efficacy/safety in dogs. If satisfactory, safety trials will be initiated in major non-target animal species. Additional work may be conducted with new candidate vaccines under laboratory conditions and on new bait types in the field.

4.5 Rabies Laboratory, Centers for Disease Control, Atlanta, USA

The Rabies Laboratory will evaluate new attenuated and inactivated oral vaccine as well as the efficacy of parenteral and oral vaccination.

5. CONCLUSIONS/RECOMMENDATIONS

In the light of the results reviewed during this meeting oral immunization of dogs could become a powerful tool for dog rabies elimination especially in countries where dog accessibility to parenteral vaccination is low or requires extensive effort.

Special attention should be given to the identification of vaccines offering maximum safety for non-target species, especially man. They represent a prerequisite for the wide use of the oral immunization technique in densely populated urban and suburban areas in canine rabies infected countries. In this context, the particular characteristics of the safety and efficacy should be evaluated for each candidate vaccine regardless of its being attenuated or recombinant.

The development and evaluation of a bait most appropriate to dogs in a given geographical zone should be given priority consideration. In particular, the bait should be locally produced in large quantities and as inexpensive as possible.

Further studies comparing the cost effectiveness of parenteral versus oral immunization (using placebo baits) should be carried out in the near future in as many socioeconomic and geographical settings as possible.

ANNEX 1

List of participants

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