

**GUIDANCE FOR RESEARCH ON
ORAL RABIES VACCINES**

AND

**FIELD APPLICATION OF ORAL
VACCINATION OF DOGS AGAINST
RABIES**

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Table of Contents

1.	Foreword	4
2.	Vaccine and vaccine bait formulation and efficacy testing	7
2.1	Determining oral efficacy of vaccine suspension in the laboratory	7
2.1.1	Dog characteristics	7
2.1.2	Vaccine titration	7
2.1.3	Administration of vaccine suspension	8
2.1.4	Immune-response	8
2.1.5	Virus challenge	8
2.1.6	Special considerations	8
2.2	Bait composition for dogs and vaccine bait efficacy testing	8
2.2.1	General considerations on baits:	9
2.2.2	Attractants	9
2.2.3	Biomarkers	10
2.3	Efficacy testing of the vaccine bait in confined dogs	10
3.	Safety requirements for oral dog rabies candidate vaccines	12
3.1	Safety requirements for modified live vaccines (MLV)	12
3.1.1	Laboratory tests in target species	12
3.1.2	Other target species	13
3.1.3	Safety for non-target	13
3.2	Safety requirements for recombinant live virus vaccines (RLV)	14
3.2.1	Laboratory tests in target species	14
3.2.2	Safety for non-target species	14
4.	Bait preference and bait distribution system testing in the field	16
4.1	General recommendations (for baiting systems and bait delivery)	16
4.2	Standardized test for determining dog bait preferences	16
4.3	Assessment of vaccine bait distribution to dogs	17
4.4	Protocols for testing and comparing of different bait distribution systems	18
4.4.1	Requirements	18
4.4.2	Preliminary experiment: Testing of vaccine-bait acceptance under field conditions	19
4.4.3	Bait distribution to dog owners in a central place	20
4.4.4	Door-to-door baiting of owned dogs	22
4.4.5	Bait distribution according to the wildlife-immunization model (WIM)	24
4.4.6	Further studies	25
5.	Dog population parameters relevant for and applied to rabies control	26
5.1.	Population size	26
5.2	Sex ratio	26
5.3	Age structure and population turnover	26
5.4	Levels of dog supervision	27
5.5	Accessibility of dogs	27
5.6	Dog vaccination coverage	27
5.7	Choice of vaccination strategy	28
6.	Risk assessment prior to release of vaccine bait into the environment	29
6.1	Vaccine strain characteristics to be considered in risk hazard description	30
6.2	Public Health Issues to be addressed by risk assessment	30
6.2.1	Probability of exposure through bait contacts	30
6.2.2	Probability of exposure to vaccine	31

6.2.3	Probability of disease occurrence	31
7.	Recommendations for the implementation of oral vaccination projects	32
7.1	General considerations	32
7.2	Release of baits into the environment	33
7.2.1	Operational objectives	33
7.2.2	Organizing field trials for the oral vaccination of dogs (OVD)	33
7.2.3	Infrastructure requirements	35
7.2.4	Preparation of field trial site	35
7.2.5	Monitoring of human exposure to vaccine and risk management evaluation	38
7.2.6	Post-baiting evaluation of the project	39
7.2.7	International cooperation	40
ANNEX 1	List of participants and contributors in alphabetical order	41
ANNEX 2	Bibliographical References	46
ANNEX 3	Dog ecology technologies and techniques	49
ANNEX 4	Categorization of individual dogs according to restriction by and dependency on humans	52

1. Foreword

Globally, almost all human deaths attributed to rabies are caused by dog bites and approximately 99.9 % of reported cases occur in Asia and Africa. It is estimated that approximately 55,000 human lives are lost to rabies each year on these 2 continents (1). Asia accounts for an estimated 32 000 annual deaths (58%) with about 20 000 in India alone (62% of the Asian mortality figure). Africa accounts for 23 000 of these 55 000 annual deaths (42%). With a total DALY score of 2 million rabies ranks next to lymphatic filariasis and intestinal parasitic infestations but ahead of leishmaniasis, schistosomiasis, sleeping sickness, onchocerciasis, Chagas disease and Dengue. More than 80% of the total population living in these regions (estimated at 3 billion people) is at risk from endemic canine rabies, and enormous anxiety and suffering is caused by the estimated 4.2 million annual bites from suspect rabid dogs. The estimated annual incidence is 1.37 /100,000 people, with 10 to 18 times more deaths occurring in rural than urban areas respectively in Africa and India. There are huge disparities in the affordability and accessibility of post-exposure treatment, levels of rabies awareness and risks of exposure to rabid dogs. These result in a skewed distribution of the disease burden across society, with the major impact falling on those members of poor rural communities. In addition an average 40% of the people bitten by rabies suspect dogs are less than 15 year of age increasing the consequences for poor rural communities.

WHO has always tried to maintain a balance between the promotion of activities for the prevention of human rabies and the control of rabies in dogs. In the human field during the past 20 years WHO has consistently promoted the discontinuation of production and application of brain tissue vaccines in humans and the use of economical intradermal post-exposure immunization regimens.

In the animal sector as dog accessibility to vaccination by the parenteral route was reported to be the major obstacle for dog rabies control in many different parts of the world since 1985, WHO promoted research on dog populations and achievable dog immunization coverage in Africa, Asia and Latin America [3-7]. Acknowledging the insufficiencies of the parenteral route for dog rabies elimination, WHO stimulated studies on oral vaccination of dogs (OVD) and the development of safer and effective vaccines and baits for OVD [8-15, 37-40]. This document is a compilation of recommendations made by the consultations on OVD organized by the Zoonoses and Veterinary Public health unit of WHO.

OVD offers new approaches promising a significant increase in the dog vaccination coverage (especially of free-roaming and poorly supervised dogs) both when applied exclusively or in combination with parenteral vaccination [6-7]. Since 1988 WHO has continuously promoted international collaboration and coordinated research in OVD through an informal group of specialists associating specialized WHO collaborating centers, researchers and official representatives of potential recipient countries, as well as pharmaceutical companies. Very early on it became evident to this group that ensuring the safety of OVD (from candidate vaccine to bait and bait delivery systems) under the specific conditions prevailing in most areas with dog rabies was a prerequisite to promoting its use in the field. OVD safety for non-target species, especially humans, has remained the center of WHO coordinated activities. The group very carefully looked at different probable and also more unlikely scenarios which could lead to human exposure to a live dog vaccine [8-13].

To better assess the likelihood of these different scenarios the group requested that all candidate vaccines be tested in immuno-suppressed animal models and for safety in non-human primates. It was further recommended that better quantitative tests be developed to measure input vaccine virus excretion and that the levels of virus excretion with time be evaluated in young puppies as the most probable excretor and transmitter of vaccine virus to humans [12-13].

The group also established guidelines for determining oral vaccine efficacy in laboratory dogs and for bait development, bait preference trials and for the evaluation of bait delivery systems in the field [16]. Three delivery systems for OVD were envisaged : the distribution of the baits to owned dogs via their owner who would collect the bait at a central location, b) the placement of baits at selected sites where they were accessible to free-roaming dogs (so-called « wildlife immunization model ») and c) distribution of baits to dogs encountered in the street (so-called « hand-out model »). The group worked on elaborating specific guidelines for implementing OVD projects and has promoted the further investigation of OVD logistics and economics.

Investigating economics of OVD is essential since it is very unlikely that all resources required for dog rabies elimination become suddenly available. The implementation of control activities will obviously remain under financial strain and require that new techniques be as cost effective as possible. When targeting certain « high risk » components of the dog population such as feral and free-roaming dogs it may be possible to accept a cost per dog vaccinated by the oral route higher than that established for a parenteral vaccination (e.g. US\$ 1 to 1.3 with 0.35 worth of vaccine [18]) as most savings accrue after rabies elimination. However, when oral and parenteral vaccination compete for the same dog (e.g. owned and restrainable segment of the population) one should expect at least comparable costs per fully vaccinated dog.

To reduce costs further and thereby open new opportunities for the initiation of large scale vaccination programmes, inexpensive and voluntary vaccine delivery systems involving communities or community leaders should be promoted. In this context, the results acquired in Tunisia by placebo bait distribution to dogs via their owners are very encouraging [17]. This method would however necessitate modifications of regulation on the delivery and application of veterinary rabies vaccines currently enforced in many countries. It should also be kept in mind, that this move might not be well received by professional associations and governments struggling to allocate often limited budgets to competing public health problems.

A number of requirements regarding safety of candidate vaccines and safety, efficacy and economics of bait delivery (using placebo baits) still remain to be fulfilled. WHO-coordinated laboratory and field research on OVD has however been fruitful and created the proper conditions for launching limited field trials in the near future.

This document aims at sharing guidance generated by the WHO specialist OVD group with field researchers, public health and animal health administrators involved in rabies control programmeme organization and implementation. The composition of the Specialist Group which assisted WHO in this endeavour varied over time. The list of contributors attached to this document as Annex 1 is a compilation of the lists of participants of each of the OVD Consultations organized on the subject. Their contribution has been very much appreciated and this document also aims at acknowledging their valuable involvement in the area. As this series of OVD Consultations spanned over more than 2 decades many of the participants have moved to new positions, some are now retired and some unfortunately have passed away. We

have kept for each of these categories of contributors the affiliations which were theirs at the time of their last participation.

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2. Vaccine and vaccine bait formulation and efficacy testing

The group defined efficacy as protection of a vaccinated animal against challenge. Immunogenicity as measured by virus neutralizing antibody response was considered only a part of the vaccine efficacy assessment.

The group felt it was important to examine the following: Vaccine efficacy when administered by instillation, efficacy of vaccines when given in a bait to caged dogs (vaccine-in-bait efficacy) and efficacy of vaccine baits in the field;

To date, several candidate vaccines (e.g. the attenuated SAG2 and recombinant V-RG viruses) have met minimum WHO recommendations as regards oral efficacy via a bait against relevant street rabies virus challenge, preferably using a well-characterized street virus of dog origin (salivary gland material) administered in a concentration sufficient to kill 80% of unvaccinated controls [10]. National vaccine standards should be met by candidate oral vaccines. It may be necessary to reassess efficacy with each significant variation of baits and bait delivery systems (e.g., bait mixed in food, etc.).

2.1 Determining oral efficacy of vaccine suspension in the laboratory

2.1.1 Dog characteristics

Test dogs should only be used once per rabies vaccine experiment. There should be at least 5 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 grouped by sex and reproductive conditions. They should be at least 6-12 months of age. 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. Routine veterinary care and husbandry should be performed according to humane conditions.

Before engaging in efficacy testing according to this outline it is advisable to consult national test guidelines (i.e. 9CFR or European Pharmacopoeia) and to adapt the protocol if indicated.

2.1.2 Vaccine titration

For Modified Live Vaccine (MLV), infective titers can be measured in mouse (MICLD₅₀/ml) or in tissue culture inoculation (e.g. TCID₅₀/ml or PFU/ml) [34]. The first dilution of the vaccine should be that obtained at the production level. If found effective, several serial dilution (e.g. tenfold, etc.) should be tested. The titer (per 1.0 ml) must be stated including the corresponding unit (see above). The diluent should be cell culture medium (e.g. PBS or Hanks without serum). Vaccine titer should be determined immediately after vaccination. Analogously, appropriate methods for the quantization of recombinant vaccines should be used. All methods should be validated and tests should include a viral standard control .

2.1.3 Administration of vaccine suspension

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 center of the tongue using a needle-less syringe; there should be no contact with tissues.

2.1.4 Immune-response

Anti-rabies serology [either *in vitro* (e.g. RFFIT, FAVN, ELISA) or *in vivo* (mouse) neutralization test] should be conducted before vaccination [34]; dogs participating in the experiment should have no detectable rabies virus neutralizing antibodies. It is suggested that the post vaccination serology begins on day 7, be obtained weekly thereafter through Day 28, on the day of challenge and 7 days after 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 an international reference serum).

The duration of antibody titers in the vaccinated dogs should be assessed under laboratory and field conditions.

2.1.5 Challenge virus and challenge

The challenge virus 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 routes and site(s) of inoculation at time of challenge should be the same as 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 caged « field » dogs should follow the protocols recommended for laboratory dogs as closely as possible.

2.1.6 Special considerations

Virus strains used in oral mass vaccination of dogs should be identifiable by standard laboratory methods.

2.2 Bait composition for dogs and vaccine bait efficacy testing

The development and the 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 inexpensively as possible.

Field trials should be conducted to better identify the advantages and disadvantages of machine-manufactured versus hand-crafted baits. Until sufficient data become available, all options with respect to bait types and means of bait delivery should remain open. Furthermore, as vaccination programmes will be planned and executed by national, provincial or local governments, adaptation to prevailing conditions at each level within a given country is required. It should be borne in mind that what is suitable for one country may not necessarily be most appropriate for another.

2.2.1 General considerations on baits:

The ideal bait should:

- immediately attract the target species;
- the shape of the bait should allow easy ingestion by all ages and sizes of dogs;
- optimize the release of vaccine into the oral cavity and to the target tissues;
- should contain the vaccine in a presentation not detracting from vaccine efficacy;
- be free from adventitious agents;
- not attract non-target species, including humans;
- be safe for target and non-target species if baits, even many, are consumed;
- protect the vaccine under field conditions;
- be economic to produce in standard form, possibly under local conditions;
- should allow the incorporation of a biomarker;
- withstand potentially extreme environmental and storage conditions;
- feature a labeling system may be required to identify producer, user, vaccine strain, batch number and expiration date

2.2.2 Attractants

An attractant should:

- present optimal stimuli (e.g. visual, tactile, olfactory/gustatory) for target species while minimally attractive to non-target species;
- be compatible with bait and vaccine and adherent to the bait;
- remain palatable for a defined period;

- withstand temperature extremes;
- be economical and possibly producible under local conditions.

2.2.3 Biomarkers

In general, markers in current use can be grouped as follows:

- a) surface markers (Rhodamine B, other dyes);
- b) tissue markers (Iophenoxic acid, etc.);
- c) calciphilic markers (Tetracycline).

Biomarkers should be:

- compatible with other bait components ;
- safe for target and non-target species ;
- detectable in target species for a defined period using technically simple, economical and locally available assay methods ;
- absent or minimally present in subject population ;
- economical to produce.

2.3 Efficacy testing of the vaccine bait in confined dogs

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

The vaccine presentation should optimize the release of 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 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 micro-encapsulated particle. Whatever the presentation it should ensure that the vaccine cannot easily be separated from the matrix and rejected. Such rejection may be via the vaccine container or rejection of large particles of the bait containing tablets, capsules etc. Following the establishment of basic efficacy, innocuity testing should commence.

Recommendations of how to carry out efficacy studies with vaccine incorporated in a bait are as follows:

- Vaccine-bait efficacy studies should be preceded by vaccine (suspension) efficacy studies

and bait acceptability tests.

- Vaccine-bait efficacy testing should follow the principles outlined for vaccine suspension testing (2.1).
- Only one bait should be offered per animal.
- Effective dose and volume should be carefully defined and recorded so that comparisons could be made with other studies, although it needs not be standardized.
- The administration of vaccine-baits should conform to the principles of bait acceptance trials described in the relevant literature.
- The administration of baits should be standardized and recorded in terms of time of day, dog feeding habits, and husbandry, etc.
- The baits should be consumed within a reasonable period of time and, if possible, the time of consumption should be recorded.
- In addition, vaccine-bait efficacy should be evaluated in terms of duration of immunity under laboratory and field conditions
- Claims of vaccine efficacy in dogs under 3 month of age should be thoroughly supported by evidence in laboratory and field dogs of the corresponding age group.
- Vaccine stability in the bait under field conditions needs to be demonstrated

3. Safety requirements for oral dog rabies candidate vaccines

Considering the likelihood of exposure of humans to oral vaccines during OVD activities due to the close association of dogs with humans, special attention should be given to the identification of vaccines offering maximum safety for non-target species, especially human beings. They represent a prerequisite for the wide use of the oral immunization technique in densely populated urban and suburban areas of canine rabies infected countries. In this context, the safety and efficacy should be evaluated according to the recommendations given below for each candidate regardless of its being attenuated or recombinant.

3.1 Safety requirements of modified live vaccines (MLV)

The candidate vaccine strain should be characterized according to classical procedures.

3.1.1 Laboratory tests in target species

- To test for possible latency, 10 dogs given 10 times the field concentration orally should be tested by appropriate techniques. Any vaccine strain leading to latency should be rejected.
- Considering that puppies 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 candidate vaccines for oral vaccination should not produce disease in dogs less than ten weeks of age when administered *per os* and intramuscularly, at 10 times the field dose. Ideally, subject dogs should reflect the intended populations at risk.
- The possibility of excretion of vaccine virus in the saliva of the animals described above should also be examined. Following vaccine application, swabs should be taken several times within the first day after oral immunization and then daily for at least 7 days. Recovery of virus in swabs should be consistent temporally and quantitatively with limited viral replication. Any virus recovered should be characterized. At the termination of the experiment, necropsies should be conducted with the examination of relevant major organ systems for the presence of virus of vaccinal origin.

As recommended previously [12], each reference or regional laboratory testing vaccine virus excretion should determine beforehand the level of sensitivity of virus detection in saliva or fecal samples for each individual test. Saliva swab samples evaluated by either animal inoculation or cell culture methods have been shown to underestimate a known quantity of reference virus by 10 to 100 viral "units" (MICLD₅₀, TCID₅₀, PFU, etc.).

Special care should be taken to avoid as much as possible neutralization or dilution of the virus potentially present in the saliva. Attention should be paid to details including swab type (natural or synthetic fibers), swab processing (swab removal versus swab being left in the medium), transport media, storage conditions (frozen, protection from light, dryness, repeated freeze/thawing, immediate use, etc.) and other considerations. Special saliva quantization systems such as the double tube system for collection of saliva may be used.

- These tests should be completed before any oral vaccination field trials in dogs are begun.

3.1.2 Other target species

Analogous procedures for other target species should be applied as described for dogs in the section above.

3.1.3 Safety for non-target species

- Local wild and domestic animal species that may take baits should be given a dose of vaccine orally equivalent to 10 times the field concentration in a volume adapted to body weight. Categories that might be more susceptible (e.g. young, pregnant) or more likely to transmit vaccine virus to humans must be included in such studies.
- 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 (e.g. the dose which is contained in a bait) orally and intramuscularly (this may require use of different virus concentrations and volumes for different species, depending on their weight and size). If the animals so vaccinated exhibit sickness or mortality from rabies, the use of the vaccine should be reconsidered.
- 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 immuno-compromised 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.

- Any further considerations of safety should be evaluated through placebo studies documenting extent and circumstances of possible human exposure to vaccines.
- Candidate vaccines should also be given by oral, intracerebral, intramuscular and other relevant routes to nude and SCID-mice or other immuno-deficient laboratory animal models.

The use of live vaccines should be discouraged when the risk of unintentional exposure to severely immuno-compromised 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 immuno-compromised individuals.

Any results obtained by the production laboratory or a WHO Collaborating Center on Rabies (regarding oral vaccination of dogs or other wild carnivores) should be corroborated by another WHO Collaborating Center on Rabies.

3.2 Safety requirements for recombinant live virus vaccines (RLV)

3.2.1 Laboratory tests in target species

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 MLV in section 3.1. 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.

Those recombinant vaccines for which innocuity in the intended species is established (as by genetic modification) may be considered of reduced virulence in other species. This in no way implies that further studies in other species should be curtailed. In addition, special studies (in case of recombinant vaccines) are indicated in species which are known to be particularly susceptible or sensitive to the parent carrier virus.

3.2.2 Safety for non-target species

Other issues than those related to the use of MLV need to be considered for the safety of RLV for non-target species and for humans. Whenever possible, the risks of vaccine virus transmission to humans (e.g. via an immuno-suppressed non-target species or a dog re-excreting the vaccine strain) should be evaluated.

As for MLV, real and hypothetical risks must be differentiated. The real risk for relevant non-target animal species can be definitely established by safety testing of this species in the laboratory. Aspects of pathogenicity of the candidate vaccine strain can and should be studied in appropriate laboratory animals including immuno-suppressed animal models, in the most relevant non-target species including wild vertebrates and, if possible, in non-human primates. 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.

A hypothetical risk is the recombination of the RLV vaccine viral genome with that of another virus, with the resulting recombinant potentially 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 RLV 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.

In the case of vaccinia vectored rabies glycoprotein vaccine (as now developed) where the rabies glycoprotein gene is inserted at the thymidine kinase position (TK-gene) in the vaccinia DNA, this vaccine may be considered non-infectious for rabies, and pre-exposure or post-exposure rabies vaccination is not recommended for persons exposed to this vaccine. Rabies risks from other recombinant vaccines must be evaluated on an individual basis as such vaccines are developed.

In summary, a safe recombinant vaccine virus candidate should:

- not acquire virulence during replication in the vaccinee;
- not be oncogenic in the vaccinee;
- should fulfill the requirements for target and non-target species established in this document
- 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.

4. Bait preference and bait distribution system testing in the field

4.1 General recommendations (for baiting systems and bait delivery)

Major carnivores for which oral vaccination techniques are desired include: dog, red fox, arctic fox, skunk, raccoon, raccoon dog, mongoose and jackal species.

Baiting system guidelines will vary widely depending upon climate, target species ethology, target and non-target species characteristics, and urban versus rural environments.

Whereas specific recommendations can be issued for rabies vaccination of dogs, this is not possible for wildlife which is composed of different animal species.

4.2 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 test 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:

- owned dogs living in the 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 head 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 WIM (Wildlife Immunization Model) is considered it may be useful to test the power of attraction of any bait candidate in a field trial first by means such as the tracking-station method or direct observation, before further use either for the evaluation of bait delivery systems or for oral mass vaccination in the field on a large scale.

Baits intended, among others, for the distribution to dog owners should be clean, easy to handle, and should fulfill the requirements made for foodstuffs so that dog owners would not find them objectionable to handle. It may be impracticable to distribute chicken head baits to dog owners. Existing artificial baits, however, as 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 head baits or Köfte baits (minced meat balls) 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 behavior and food preferences.

Although biomarkers are an effective way of estimating bait uptake, currently available biomarkers do not give a clear indication whether the animal has been immunized or not but rather reflect contact with the vaccine.

4.3 Assessment of vaccine bait distribution to dogs

The different methods include:

- door-to-door oral vaccination of owned dogs;
- presenting baits directly to dogs whether owned or unowned on the street ("hand-out" model);
- procuring owners/caretakers with baits at central sites for feeding to their dogs at home;
- placing baits at sites known to be visited by free ranging dogs ("wildlife immunization model or WIM").

All of the above vaccine bait delivery methods can be used in conjunction or not with parental vaccination.

Factors that will help determine the most appropriate bait and bait delivery systems in a given area include bait acceptance rates by target and non-target species, socio-cultural acceptance and efficacy of the delivery system, and the economics associated with programme implementation.

It is therefore recommended that bait development and evaluation continue for both industrially-manufactured and hand-crafted baits, until data permit formulation of specific guidelines for control programmes implementation. Additionally, innovative or novel approaches to the problems should be encouraged as some options have not yet been investigated.

Bait delivery tests should be undertaken in as many different areas as possible. In each situation the utility of various bait distribution strategies (with placebo, no vaccine) should be evaluated according to the principles described below [16] to determine the most effective method appropriate for the described dog ecology parameters. When doing this, consideration should be given to social and cultural human factors, human density, and dog population structure, dynamics, and feeding patterns. It is further recommended to include cost-effectiveness of the different strategies (door-to-door-baiting, central-point distribution, WIM, parenteral vaccination and combinations of different vaccine delivery systems) in future field trials using appropriate evaluation methods. An economist should assist in designing the evaluation methodology.

Standardization of field trials should be improved. Categories of dogs should be defined on an operational basis (e.g. owned dogs, dogs accessible by parenteral vaccination, free-roaming dogs etc.). To compare the results of one study to another, criteria or allocating a dog to a given category needs to be precisely described (Annex 4). In order to study certain aspects of bait delivery, methods recommended by WHO should be revised for estimating dog population parameters.

Data already available suggest that as an adjunct to parenteral vaccination, bait distribution to dog owners at central points and by door-to-door distribution or at mobile points may significantly increase the overall vaccination coverage.

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 WIM 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 owned dogs.

4.4 Protocols for testing and comparing of different bait distribution systems

This section provides guidelines for the elaboration of a research design for testing various vaccine bait delivery systems in the field. It is intended for research workers wishing to study the feasibility of oral immunization of dogs by comparing different delivery systems in order to select the delivery system or the combination of systems best adapted to for routine operation in prevailing local conditions.

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. The various protocols for oral immunization suggested below may be tested in isolation or in combination (e.g. door-to-door baiting combined with bait distribution according to the WIM) and could also be evaluated in association with conventional parenteral vaccination strategies (e.g. door-to-door or central mobile points parenteral vaccination campaigns associated with bait distribution according to WIM).

This section aims also at providing researchers with a basic study concept, the use of which should facilitate comparison of the different bait delivery models.

4.4.1 Requirements

Bait development and bait selection

For the proposed study, whichever type of bait is selected should correspond to the requirements established (2.2). To test the preference of the dogs for different baits, WHO recommendations should also be followed [10].

Markers

To evaluate the accessibility of baits to dogs by a method not involving direct observation, a

systemic marker should be used instead of vaccine. The availability of a systemic marker and the necessary laboratory facility for marker detection in animal tissues is essential for conducting the experiments described below.

The use of a topical marker in place of vaccine is not compulsory although it may provide immediate visible information on the contact between the oral mucosa and the content of the vaccine container.

If systemic and topical markers are used it should be ensured that no undesired interaction between these substances will influence the outcome of the experiments.

Systemic marker

It is assumed that a serum marker will be used (e.g. Sulfadimethoxine [SDM] or Iophenoxic acid [18-19] but any other kind of systemic marker which does not require post-mortem sampling for the detection may also be useful. The vaccine container should be filled with the marker and inserted into the bait. Preferably the marker should have a rather long half-life.

Topical marker

Topical markers are Rhodamine B, Methylene Blue etc. [20-21]. The vaccine container should be filled with the marker.

Study sites

It is proposed that villages with the following characteristics be chosen for the study:

- 5000-10'000 inhabitants;
- more than 500 dogs;
- knowledge of the exact number of inhabitants and/or the number of households;
- available maps (or the area should be easy to map);
- easily-defined boundaries.

4.4.2 Preliminary experiment: Testing of vaccine-bait acceptance under field conditions

This experiment will provide information about:

- bait acceptance
- contact with the « vaccine » (e.g. the systemic or topical marker contained in the vaccine chamber).

Material

Baits with serum marker (at least 150-200 baits), questionnaire forms, collars and/or dye pens, camera, maps, chronometer, material for phlebotomy, etc. should all be prepared in advance.

The minimum number of persons required for field work is 2.

The systemic marker to be used for the following three studies (4.4.3, 4.4.4, 4.4.5) should be

tested in this experiment. As an additional control, a topical marker could be used in order to have an immediate visible indicator of the mucosa-« vaccine » contact.

Procedure

This preliminary experiment should be performed as follows:

- 1) Give baits to a minimum of 100 owned dogs selected at random ;
- 2) Identify the reference household of each dog (on a map) ;
- 3) Mark by placing a collar on the dog (or by using dye), by making a picture or use two techniques to mark each dog to which a bait is offered ;
- 4) Note all important parameters concerning behavior and bait acceptance (length of test period ; time span until physical contact with the bait occurs, proportion of the bait consumed, whether the bait is chewed or not chewed, presence of dye markers on mouth or teeth (if a topical marker is used) etc.) [10];
- 5) By means of a questionnaire form, collect additional information on number of family members ; number of dogs per household (those present or absent at time of visit) ; their sex and age and whether the dogs are free-roaming or confined. The questionnaire should be short and easy to answer ;
- 6) On the following day(s), take a blood sample of each marked dog ;
- 7) Check blood samples for marker.

As all dogs offered a bait are marked, including those which did not even come into physical contact with the bait, some blood samples will serve as negative controls for the marker. However, the collection of a large number of blood samples might turn out to be too time consuming. If this is the case, marking and blood sampling could either be limited to dogs which had physical contact with the bait or to dogs which had (mucosal) contact with the topical marker.

4.4.3 Bait distribution to dog owners in a central place

This experiment should allow the estimation of:

- the size and density of the population of owned dogs (dogs per square kilometers, number of dogs per human beings, number of dogs per household) ;
- the percentage of households with and without dogs ;
- the percentage of dog-owners motivated by the information campaign ;
- the percentage of dogs successfully « vaccinated » by this method (detection of topical or systemic marker);
- the mean number of baits needed to « vaccinate » one dog by this bait distribution method ;
- the costs and benefits.

Material

The following should be prepared or arranged in advance: means of transport, loud-speaker, maps, chronometer, baits prepared with serum marker (> 500 baits), blank baits (without marker), material for phlebotomy, dog catching facilities (anesthetic, blow-pipe, dart-gun, etc.), questionnaire forms, etc.

The minimum number of people required for field work is 3.

This experiment should be carried out in the same village as the preliminary experiment (4.4.2).

Procedure

The experiment should be performed as described below:

- 1) Provide advance notice to inhabitants as to the date, time and place of bait distribution. Clearly advise them to come to the distribution center without dogs. Announcements should be made and instructions provided using loud-speakers. The information campaign should be made by car, motor-cycle or bicycle.

The information campaign should be considered an integral part of the study. Consequently, all details must be recorded (time required, route taken, kilometers driven by car, number of announcements per minute, costs etc.);

- 2) Distribute baits to dog-owners in one or two central places (the number of bait-distribution sites depends on the structure of the village). Give to each dog-owner as many baits as she/he declares having dogs (1 bait per dog). Take into account, that the dog-owner might not declare puppies. If dog owners from outside the test area request baits, hand out baits without biomarker.
- 3) Note the number of baits distributed, number of dog-owners, number of baits distributed per dog-owner, as well as the dog-owner's names and the total time spent for bait distribution if possible.
- 4) On the following day(s), take blood samples either from :
 - a) all dogs in all households, or
 - b) all dogs in a representative sample of households (at least 100 dogs or more if possible), or
 - c) a representative sample of the owned dog population in a representative sample of households (please bear in mind the level of confinement and do not always select those dogs which are easiest to handle! Investigate at least 100 dogs or more if possible).

If possible, try to use method a). Use b) only if maps are available. Number each household on the map. Select at random the order in which these houses are to be visited. Follow this order until blood samples have been taken from a sufficient number of dogs. If different sample-selection techniques are used, make sure that each household has the same probability of being chosen. It is recommended to avoid using method c).

If blood samples cannot be taken from a given dog, make sure that it can be identified (photographic marking, etc.) and try again later on the same dog. Use a dog catching device if necessary;

- 5) Using a questionnaire, collect additional information on the number of family members;

number of dogs per household (both those present and absent); their sex and age; number of free-roaming and confined dogs.

The questionnaire should be short and easy to answer;

- 6) Check serum samples for marker.

4.4.4 Door-to-door baiting of owned dogs

This experiment should be considered an integral part of the third experiment described below (bait distribution according to the WIM - see section 4.4.5). It may, however, be carried out independently from the WIM experiment but the WIM experiment may not be carried out independently of this one.

This experiment will allow the estimation of:

- the number of owned dogs (per square kilometers, per household , ratio of dogs : human beings);
- the number of households with dogs;
- the total number of dogs (per square kilometers, ratio of dogs : human beings, ratio of owned dogs : ownerless dogs);
- the number of ownerless dogs (per square kilometers, ratio of dogs : human beings, ratio of owned dogs : ownerless dogs);
- the total number of dogs « vaccinated » by the door-to-door distribution method;
- costs and benefits.

Material

The following should be prepared or made available in advance : placebo baits (without marker), maps, a chronometer, two types of collars (of different color) and/or two types of dye pens (of different color), questionnaire forms, material for phlebotomy, dog catching facilities (anesthetic, blow-pipe, dart-gun etc.), binoculars, etc.

The minimum number of persons required for field work is 2.

As a further control, a topical marker (for example Rhodamine B) could be used to have an immediate visible indicator of liquid-mucosa contact.

Do not carry out this experiment in the same village as the preliminary experiment.

Procedure

The experiment should be performed as described below:

- 1) Using the door-to-door method, visit all households, identifying each one on the map;
- 2) Use baits without marker if the following experiment (4.4.5) is to be carried out, and with marker if experiment 4.4.5 is not to be carried out. Give the baits to the dog-owner who should then be present one to each of his dogs;
- 3) Note all important parameters concerning behavior and bait acceptance (see

- « preliminary experiment » 4.2.2);
- 4) Collect baits or parts of baits untouched after a certain time limit (2-5 minutes);
 - 5) Mark with a collar and/or dye pen etc. every owned dog which has accepted the bait;
 - 6) By means of questionnaire, collect additional information on the number of family members; number of dogs per household (both present and absent); their sex and age; number of free-roaming and confined dogs; whether the dogs can be observed from the street.

The questionnaire should be short and easy to answer.

The rate of loss of the collar and the turnover of the dog population give an indication of the time period during which the bait-distribution to dog owners must be completed. To limit possible bias it should be done within a few days;

- 7) Estimate the total dog population by a capture-mark-recapture method [22-26]. Re-observation should be carried 3 to 6 times (2 re-observations per day) ;
- 8) If a serum biomarker was used, take blood samples on the following day(s) either from
 - a) all dogs in all households, or
 - b) all dogs in a representative sample of households (at least 100 dogs or more if possible), or
 - c) a representative sample of owned dogs (again, consider the level of confinement and do not always select those dogs which are easiest to handle) in a representative sample of households (at least 100 dogs or more if possible).

If possible, try to use method a). Use b) only if maps are available. Number each household on the map. Select at random the order in which these houses are to be visited. Follow this order until blood samples have been taken from a sufficient number of dogs. If different sample selection techniques are used, make sure that each household has the same probability of being chosen. It is recommended to avoid using method c).

If a blood sample cannot be taken from a given dog, make sure that it can be identified (photographic marking, etc.) and try again later on the same dog. Use a dog catching device if necessary.

Please note that blood samples must be taken from marked as well as unmarked owned dogs;

- 9) Mark every owned dog from which a blood sample is taken, with a collar or dye pen, even if it is already marked. In this case, use the second color for marking.

This step is necessary only if the following experiment (4.4.5) is to be carried out;

- 10) Check blood sample for marker.

Blood samples should be taken within a short time-period determined by the half-life of the marker used.

If a second serum marker is available, the preliminary experiment could be included into this experiment. In this case, baits prepared with the second marker would replace baits without marker.

4.4.5 Bait distribution according to the wildlife-immunization model (WIM)

This experiment should be performed in combination with the preceding experiment (4.4.4) and should be carried out in the same village. It will allow the estimation of:

- the percentage of owned dogs « vaccinated » by WIM ;
- the percentage of the total dog population « vaccinated » by WIM ;
- the percentage of the total dog population « vaccinated » by WIM and the door-to-door bait-distribution method ;
- costs and benefits.

Material

The following should be prepared or arranged in advance: baits with serum marker (the half-life of the marker used should not be too short), maps, collars (of the second type used in the preceding experiment) and/or dye pens, material for phlebotomy, dog catching facilities (anesthetic, blow-pipe, dart gun etc.), binoculars, etc.

The minimum of persons required for field work is 4.

Procedure

- 1) Perform steps 1 to 7 of preceding experiment (4.4.4) and then distribute baits with markers following pre-established scheme (e.g. along transect lines or along both sides of streets) within the study area. Distribute a quantity of baits approximately 3 times the estimated total number of dogs. Baits distribution is preferably done in late afternoon/early evening after the peak of maximum human activity. Collect all baits not consumed within 18 to 24 hours.

The distribution of baits is an integral part of the study, consequently all details must be recorded (time required, route taken, kilometers driven by car, number of baits distributed, sites where baits were placed etc.) ;

- 2) Perform steps 8 to 9 of preceding experiment (4.4.4) and then take blood samples from the maximum number of unmarked dogs roaming the streets (both marked and unmarked). Mark every dog from which a blood sample is taken with a collar or a dye pen.
- 3) If dogs are killed to collect samples, the killing should not be done by using poison-loaded baits -to avoid certain sampling bias and adversely affecting any further oral vaccination campaigns) ;
- 4) Check blood samples for marker.

4.4.6 Further studies

These experiments can also be used to test other combinations of vaccination strategies

including parenteral vaccination. As an example, bait delivery by WIM could be preceded by a parenteral mass vaccination campaign in central vaccination sites or by door-to-door vaccination instead of immunizing owned dogs orally.

Results of these experiments would ultimately allow the selection of one of, or a combination of several vaccination techniques (oral, parenteral). Further studies would aim at optimizing the selected strategy by modifying the protocol, for example changing the number of baits distributed per square kilometer, number of information announcements made, increasing the time limit for bait consumption and so on. The costs and benefits of parenteral-oral vaccination strategies could then be compared to the oral-oral strategies described above.

New risk assessment models for human exposure during field application of vaccine baits should be made and validated with field data from the specific location.

5. Dog population parameters relevant for and applied to rabies control

Six parameters are necessary to characterize the demography of a dog population when planning and/or surveying a vaccination programme against canine rabies. These are:

- (a) population size, e.g. density per ha or square kilometer or humans : dog ratio
- (b) sex ratio ;
- (c) age structure ;
- (d) annual population turnover, e.g. proportion of new dogs entering the population ;
- (e) levels of dog supervision and
- (f) accessibility of dogs (e.g. to owners, to interventions).

5.1. Population size

A general deficiency of most rabies control projects is the inaccuracy of the estimates of the actual dog population size. It is neither difficult nor expensive to combine initial vaccination schemes with marking of vaccinated/captured dogs and to assess through visual recapture techniques the true population size supposing that marked and unmarked dogs have equal chances of being recorded, so that the bias can be estimated. Accurate dog population estimates form the basis for marshaling human and material resources, and provide the base-line data for subsequent vaccination coverage and economical population control programme evaluation. Annex 3 provides technologies and techniques for dog ecology studies [28].

5.2 Sex ratio

Sex ratio can be determined from information collected in questionnaire surveys and/or at the time of vaccination. If differences in the two findings are observed, this may indicate sampling biases which should be investigated. Sex ratio data will be needed for understanding population dynamics and canine reproductive patterns.

5.3 Age structure and population turnover

Data regarding age should be collected in questionnaire surveys and/or at time of vaccination. Age should ideally be determined by months/years, but it may be more practical to divide the population into more easily distinguishable age groups: puppies (e.g. ≤ 3 months, or 0 - 6 weeks [not weaned]), juveniles (> 3 months to 1 year, or > 6 weeks [weaned] to 1 year) and adult dogs (≥ 1 year). The size of the unvaccinatable population (under 6 weeks or 3 months of age depending on the efficacy of the vaccine, respectively), of the juvenile dogs entering the vaccinatable population, and the proportion of the adult population which is unvaccinated are all data needed in planning vaccination strategies. The annual recruitment into the dog population of unvaccinated dogs may be estimated from the proportion of the population between 3 months (or 6 weeks) and 12 months of age. This is important in determining the interval between vaccination campaigns.

5.4 Levels of dog supervision

Vaccination strategies are closely related to the level, daily duration and purpose of dog supervision. Information on this important parameter can be obtained through questionnaire surveys as described above, and from the WHO Guidelines for Dog Rabies Control [27]. Simplified dog accessibility studies requiring less than one day for two persons may also reveal essential data. The level of dog supervision must, however, be examined during the first phase, or pilot project, of a large-scale rabies vaccination campaign. Preparation of a vaccination campaign (e.g. through schools), organization (e.g. neighborhood centers or house-to-house visits) and implementation (time of day, incentives) may have a considerable influence on the proportion of animals presented as well as revealing varying levels of dog supervision.

In view of the importance of this parameter, the consultation strongly recommends that great emphasis be placed on this question , along with dog accessibility studies in phases of health systems research in relation to rabies control conducted during the implementation of a control programme.

5.5 Accessibility of dogs

Accessibility of dogs defines the percentage of dogs in a given population which can be caught by a person without special effort. The vast majority of those dogs are considered accessible for parenteral vaccination. House-to-house surveys can easily reveal essential data if one assumes all dogs to be accessible, as when referenced households are identified or when collars or other marks indicate responsible dog ownership, or when dogs react in a friendly way to the touch of a stranger (e.g. the interviewer). Dog accessibility studies in several countries (e.g. South-east Asia, North and South Africa etc.) reveal a rate of over 85% of that population. Health system research is required on community participation needed to attain this rate in vaccination programmes.

Accessibility rates can also be used to obtain information on the proportion of unrestricted and non-accessible dogs which may jeopardize rabies control efforts. Semi-restricted family dogs, neighborhood dogs and truly unrestricted dogs, such as categorized in Annex 4 and WHO/Rab.Res./88.25 [28] may be considered the high-risk component of the dog population in terms of rabies infection and are thus candidates for oral rabies vaccination.

5.6 Dog vaccination coverage

A total population vaccination level of 80% is desired. Following each campaign the coverage can be determined by comparing vaccination records to dog population size, or by a separate survey. The vaccination coverage is especially important in juvenile dogs recruited into the vaccinatable population. Repeat vaccination campaigns should be conducted when population vaccination levels drops below 60%. In many areas repeat campaigns must be conducted annually and special efforts made through motivational campaigns to have puppies presented for vaccination even before reaching three months of age.

The marking of dogs is important, using as long-lasting a method as possible and definitely until evaluation of vaccination coverage has been completed. Economy of such marking is

important if the government has to provide the markers as part of the rabies control programme. Dye or paint on the forehead, collars made of plastic tubing threaded with light wire or of nylon or polyethylene rope may be considered according to local conditions. It is important that collars are not harmful, not easily removed and have no alternate consumer utility.

5.7 Choice of vaccination strategy

Information on dog ecology and population dynamics is also needed in order to decide on whether to include oral vaccination into the rabies control programme and, if that is the case, to develop effective means of bait delivery. This includes acquisitions of data from proposed rabies control areas relative to

- the proportion of owned dogs in the entire dog population and their levels of confinement (from totally restricted to permanently free-ranging);
- the proportion of unowned dogs and where they are living;
- the annual dog population turn-over so that along with vaccination rates, the frequency of campaigns for parenteral vaccination and/or vaccine bait application can be determined. Sound data are needed to determine the number of new susceptible dogs that annually enter the population;
- the percentage of the dog population which are accessible and available for parental versus oral vaccination;
- the size of dog populations and the logistics associated with means of transport, movement and numbers of available vaccination teams, baits, and other supplies will also determine the efficacy of vaccination, both parentally and orally, or in combination.

6. Risk assessment prior to release of vaccine bait into the environment

Risk assessment consists in estimating the probability of the occurrence of adverse events and the consequences if adverse events occur. In the case of the environmental release of veterinary biologics, this requires first the identification of risk factors inherent to the agent under consideration and all potentially hazardous situation or situations (scenario or scenarios) that might occur in animals and humans as well as the estimation of the possible impact of this release onto the environment (release assessment). In hazard identification it is important to consider the possible chain or chains of events leading to their occurrence(s). In addition it necessitates the differentiation between independent and dependent events as well as the determination of the factors which may influence a given scenario.

The results of hazard identification and release assessment should be integrated into a risk statement comprised of (a) likelihood rating (low, medium, high likelihood of occurrence), (b) consequence rating (consequences of adverse event are not severe, moderately severe, severe), and (c) degree of certainty rating (event is certain, moderately certain, uncertain to occur), such as proposed by the APHIS/USDA document on risk assessment for Veterinary Biologics [29]. Taking into account these ratings, risk ratings (probabilities) are then calculated according to the multiplication rules of independent probabilities. In some situations it may not be possible to calculate the risk of a given event as necessary data may not be available or only fragmentary. In these instances the likelihood of a certain events and of the process leading to the occurrence of the hazardous situation may only be subjectively estimated as low, medium or high. The risk assessment document is completed by a risk discussion and suggestions for risk management.

Dogs are very closely associated with humans, especially with children, in a majority of cultures. Non-routine activities such as parenteral or oral vaccination of dogs draws much attention from the public and interference of humans with baits has to be expected. 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. For example, during the fox rabies control effort of 1992 to 1994 in France, only 70 contacts were reported for approximately 4.4 million rabies vaccine baits distributed over farm- and woodland (1 contact for 630 000 baits) [30]. For the raccoon rabies control programmes in New York State in 1997 and 1998, exposure rate of 4 - >18 human contacts per 10.000 baits distributed were reported (1 exposure per 555-2500 baits distributed) [31]. In comparison, during limited dog bait delivery system testing in human dwellings in Tunisia [17, 32], 25 human contacts with bait matrix were observed for 314 baits distributed according to a dog-owner participation-based delivery system (1 contact per 12.5 baits), compared to 32 contacts for 1168 baits placed along transect-lines according to the WIM (1 contact per 36.5 baits).

During oral dog vaccination campaigns, the most obvious scenario is the direct exposure of a person through handling of a vaccine bait. In this context, the exposure of potentially immuno-compromised people is of special concern, since host specificity, tissue tropism and phenotypic characteristics of the vaccine strains might be changed in such an environment. When correlating the percentage of the total population exposed to baits during the Tunisian placebo field trials (approximately 1.5% of the total population) [17, 32] with WHO's 1997 adult-AIDS/HIV prevalence rates [33] of countries under consideration for oral vaccine trials, theoretical exposure of potentially immuno-compromised people could be about 2 per

1000 in South Africa, 2 per 10 000 in Thailand and India, and in the order of 2 per 100 000 in Mexico and Tunisia. Exposure may also occur indirectly through contact with a freshly vaccinated dog or a non-target species, competitor of the dog for baits (e.g. a cat). Other, more complicated scenarios could be evaluated: such as the transmission via an immuno-suppressed animal species of a strain which might differ from the parental vaccine strain.

As safety for non-target species, especially humans, is a major concern of the group, estimates should be carried out on the probability of non-target (and especially, human) exposure to the vaccine and possible untoward consequences. The risk assessment should take into account the following elements:

6.1 Vaccine strain characteristics to be considered in risk hazard description

- Character of parent strain of conventional attenuated strains, recombinant microorganisms or deletion mutants (donor and recipient genes);
- identity, purity and genetic marker of vaccine strain ;
- potential microbiological contaminants ;
- nature, site, degree and stability of attenuation ;
- host range specificity and tissue tropism,
- virulence and effect of overdosing for target and non-target animals ;
- horizontal gene transfer/recombination potential ;
- shed/spread capabilities and survivability of the microorganism in the environment ;
- environmental and geographical distribution ;

6.2 Public health issues to be addressed by risk assessment

- probability of human exposure
- pathogenicity of the parent strain in humans
- virulence of the vaccine microorganism in humans
- possible outcome of human exposure

The following issues are particular to the oral vaccination of dogs and affect the probability of human exposure :

6.2.1 Probability of exposure through bait contacts:

- vaccine delivery system (e.g. the "wildlife immunization" model may lead to an increased rate of contact versus the "door-to-door" model);
- type of bait and attractiveness for non-target;
- bait density and densities of non-target species (including humans).

6.2.2 Probability of exposure to vaccine:

- for direct exposure: types of vaccine container and vaccine formulation (liquid/lyophilized) may play a role;
- for indirect exposure (via a target or non-target animal) : the species involved, its age

- and immunological status may be important ;
- level and duration of excretion of virus in target and non-target animal species;
- survival of the vaccine strain in the environment.

A number of factors may influence the above risks; for instance the level of public awareness at time of bait release, the cultural factors influencing the rate and nature of contacts between humans, the target species and other companion animals; the prevalence of immunodeficiency syndromes in the animal population etc.

6.2.3 Probability of disease occurrence:

- type of vaccine and type of disease;
- undetected/unreported exposure;
- availability/unavailability of specific treatment;

Probabilities will ultimately have to be compared to the probability of natural occurrence of rabies in the area where the use of the technique is being contemplated. In many countries, surveillance of rabies is insufficient and no reliable official data are available. According to WHO estimates, between 5 to 10 human cases per million inhabitants are reported in hyperendemic canine rabies infected areas. Risks associated with the introduction of the technique should remain far below the risk of acquiring the disease without its use.

The group recommended that each country collects the information/data required for the calculation/evaluation of these risks by conducting placebo trials to test delivery systems and magnitude/nature of contacts between baits, vaccine containers, vaccines and non-target species especially humans.

7 Recommendations for the implementation of oral vaccination projects

7.1 General considerations

It is the responsibility of the individual countries to study the opportunity of introducing oral vaccination in their rabies control strategy. The potential role of oral vaccination of dogs should be investigated and considered only after conscientious application of traditional control measures (such as establishing or strengthening the surveillance of rabies and vaccinating dogs by the parenteral route) have yielded less than optimal results from the epidemiological and economical points of view. If countries contemplate the use of oral vaccination of dogs (OVD), the following qualification criteria should be considered:

- Dog rabies is endemic;
- a monitored dog rabies vaccination programme by parenteral vaccination is in place for the last 5 years and is permanently evaluated;
- commitment of the authorities is demonstrated by allocation of sufficient annual budget for the operation of the rabies surveillance and control programme;
- there is a network of biomedical services and diagnostic laboratory capacity (using the standard IF techniques) established in the country and historical data on human and animal rabies cases for at least the 5 previous years are available;
- dog demography information (e.g. population size estimates, density, distribution, age structure, turnover etc.) is available.

In addition, WHO staff and/or staff from relevant WHO Collaborating Centers should be closely associated with designated national authorities or independent national scientific committees when pilot research projects using oral rabies vaccines in dogs are considered.

The national team in charge of rabies control should establish the working plan for oral vaccination projects if such projects are initiated. This team should include specialists of dog ecology or acquire capabilities on this matter before starting the planning of any field trial. The population or sub-population of dogs that should be the target of oral vaccination should be identified and a strategy to reach these animals should be elaborated. The first steps of this strategy could be:

- to select one or several candidate vaccines. These vaccines should fulfill the requirements for safety and efficacy described in sections 2 & 3 of this document; Vaccine efficacy may be determined in local captive dogs administered candidate vaccine orally. The vaccine should also fulfill national requirements regarding the introduction into the country, even for experimental purpose.
- to test vaccine safety on major local non-target species competitors for baits which has (have) not already been tested for safety with the selected vaccine(s);

- to choose an already available bait or to develop a new one according to the local conditions and the method of bait delivery according to the population(s) or sub-populations of dogs which are targeted;
- to study through placebo trials the extent and circumstances of possible human exposure to the vaccine(s);
- to evaluate the acceptability of the chosen bait(s) in the target population; Before a vaccine-containing bait is used in the field its acceptance should be established in placebo baiting trials;
- before undertaking any field trial, to provide sufficient information to the public so that, in general, public support and cooperation is elicited.

7.2 Release of baits into the environment

7.2.1 Operational objectives

Provided a safe and effective oral vaccine is available, operational objectives should be determined by national authorities. These may include such options as:

- aiming at rabies control versus elimination of the disease;
- conducting a programme at national level or limited in scope to a province or some communities;
- combining oral and parenteral vaccination (carried out concurrently or sequentially), or launching an immunization programme solely based on an oral vaccine.

Availability and costs of oral vaccines, whether obtained in bulk, capsules, sachets, blister packs, or supplied within manufactured/industrial baits, will influence to some extent the selection of the best option.

7.2.2 Organizing field trials for the oral vaccination of dogs (OVD)

Project planning must precede actual field applications of baits, and related administrative activities will vary in structure and detail depending upon political and other variables. Planning and organization are vital to the success of the programme.

Project proposal

The field application of oral immunization against rabies should be based on a comprehensive plan which describes the objectives, justification, technical and organizational details and budgetary requirements of the project and defines the responsibilities of the collaborating institutions. A project proposal must include the:

- Identification of a team coordinator;

- Establishment of a budget and securing of adequate funding covering human resources, supplies and logistics requirements for completion of the trials;
- Elaboration of experimental protocols including study background, detailed description of materials and methods including vaccine to be used, and expected outcome;
- Determination of the duration of trial and baiting periodicity (i.e. number of baiting campaigns performed during the trial);
- Final cost-benefit analysis to prepare if favourable a larger scale OVD campaign.

The proposal should be distributed to concerned institutions well in advance for consideration. A scientific committee should evaluate the project. Upon request, WHO may help in providing the necessary expertise.

Responsibilities

Responsibilities for project implementation should rely upon the existing agencies, preferably inter-ministerial commissions or an Advisory Committee. Their responsibilities will be to provide guidance and coordination for field programmes within a state or country; to assume responsibility for the programme activities; to act as liaison between other activities; and to interact with news media and the public on issues relating to the project.

Budget

Adequate funds should be ensured before initiation of any field projects. These must also include funds for post-baiting evaluation and surveillance during a minimum of three years.

Legal aspects

The principles for the voluntary release of live rabies vaccines for oral vaccination of dogs into the environment should be followed. 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 of live rabies vaccines for oral vaccination of dogs into the environment.

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 to national legislation (and international requirements). These authorities advised by an independent national scientific committee (composed of national and if necessary, international experts) authorize introduction and use of product(s). Producers should make known the characteristics of the product and carry out necessary experiments satisfying minimum requirements established at national and international levels. Research workers and project designers should assess the product and identify potential hazards and evaluate related risks associated with its introduction in the environment.

International organizations especially WHO (including its network of collaborating

laboratories) has a major role to play in collaborating with governments in (a) assessing the risks associated with the use and application in the field of each type of product (e.g. modified live vaccine and recombinant vaccine), (b) identifying efficacy and safety requirements for each type of product and (c) defining criteria for its distribution in the field. WHO should also inform governments and the scientific community of any new developments in this field.

7.2.3 Infrastructure requirements

Field services

Medical and veterinary practitioners should be aware of the campaign so as to take appropriate measures in case of accidental exposure to the vaccine. An advisory group should also be established at central level.

Laboratory services

Sampling of specimens should be carried out under appropriate conditions. Laboratory facilities should be readily accessible in order to monitor vaccine safety. Laboratories must be able to carry out the tests for evaluation of the bait uptake, and seroconversion rates and to diagnose rabies.

Epidemiological investigation group

Specialists should be assigned to the campaign to investigate the epidemiological situation both in man and animals before, during, and after the implementation of the project, and should report to the responsible authorities on a regular basis.

7.2.4 Preparation of field trial site

Select site

Areas should be selected where canine rabies is endemic or epidemic. Covert rabies epidemiology in the wildlife population should be borne in mind and investigated where indicated. Preferably communities should be chosen where cooperation can easily be mobilized. Community information and education should be carried out prior to initiating the trial.

If several areas could be selected, priority should be given to those surrounded by natural barriers and/or where one can rely upon community cooperation and logistic support. The rabies situation in neighboring areas should also be taken into account. The selected areas should be readily accessible to central government veterinary/medical services.

Size of area

In the case of dog rabies, the size of the area depends upon dog population size (500 dogs or more), dog ecology, and the human-dog bond.

Epidemiological considerations

Areas should be selected according to the frequency of canine rabies or the number of dog cases and human exposures. In contrast to wildlife vaccination campaigns, dog vaccination campaigns can be initiated at any time. Certain periods may, however, prove more conducive to success than others.

Estimation of canine population size

A map of all streets and houses 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 (see Annex 3).

Estimation of 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 (Annex 4). 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 (Annex 3).

Determine optimal strategy

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

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 baits when they are not within their accustomed territory. Whether baits should be handed to dog owners in order to be fed to dogs is mainly a question of risk 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 parenteral campaign can be reached. These animals are generally not easily accessible, and if so, they may refuse to consume bait in the presence of man.

With the presently available modified live (MLV) and recombinant live (RLV) 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 risk 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.

One of several distribution methods described in section 4.4.3 to 4.4.5 or a combination thereof should be selected:

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), accessible to street distribution.

With an oral vaccination campaign most of the non-vaccinating 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 neighborhood centers.

Preparation of community participation

In order to elicit community participation the consultative group recommends that:

The Quorum process should be thoroughly adapted and modified to meet socio-ecological conditions in communities and countries where (a) children are at risk of being exposed to rabies, and/or (b) other means of interactive learning through neighborhood rabies mobilization are either not feasible or only partly practical.

Other formulations derived from experience in participatory research, informal education and community participation should be adapted to the informational, organizational implementation and evaluation needs of community-based rabies control and maintenance.

Community participation principles should be applied to every phase of rabies control and integrated with intersectorial collaboration in health systems and operational research programmes for rabies control. For this purpose, the following research needs have been identified :

- Community participation should be assessed as a method for instituting community rabies control programmes whose first objective is the creation of a self-sustaining process of community-based maintenance of rabies control, rather than the current planning emphasis on an expensive attack phase ;
- the usefulness of community members participation in rabies control should be thoroughly investigated ;
- when vaccine is unavailable, the potential for community participation in helping to

create community-specific strategies for raising the average age, health and stability of dog populations should be investigated.

- Participatory research should be employed in assessing the social acceptability of newly-introduced methods of rabies control and maintenance.

7.2.5 Monitoring of human exposure to vaccine and risk management evaluation

The oral vaccination projects that have been carried out have shown that people, especially children, take 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 people with baits designed for dogs must be minimal. Hand-feeding of baits by vaccination teams, or handing-out baits to dog-owners under the observation of vaccination teams, 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.

Setting up surveillance systems to detect human contact with vaccine and/or bait and establish rules that specify how to deal with, document, and follow-up cases of human exposure to the vaccine (e.g. how long dogs that were in contact with vaccine be isolated from humans) is of utmost importance.

There is no evidence of salivary excretion of oral rabies vaccines, especially for V-RG and SAG2, but due to the limitations of the detection methods, contact with dogs that have been administered oral vaccine should be avoided or minimized, e.g. for 1 or several hours.

Monitoring human exposure to MLV

An intense surveillance system should be established to detect any possible human exposure to vaccine. Humans accidentally in 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. Pre- and post exposure vaccination schemes should follow existing WHO recommendations (35).

Monitoring human exposure to RLV

In the case of vaccinia vectored rabies glycoprotein vaccine (as now developed) where the rabies glycoprotein gene is inserted at the thymidine kinase position (TK) in the vaccinia DNA, this vaccine may be considered non-infectious for rabies, and pre-exposure or post-exposure rabies vaccination is not recommended for persons exposed to this vaccine. Persons exposed to the vaccinia recombinant rabies virus should however be followed-up. Paired sera should be obtained following the exposure and afterwards (e.g. 30 days). Treatment should be symptomatic if illness occurs. Appropriate samples should be obtained for virus confirmation

if lesions develop. WHO collaborating centers should be contacted for assistance. Risks from other recombinant vaccines must be evaluated on an individual basis as such vaccines are developed.

Monitoring of domestic or wild animals

Any rabies virus isolated from animals in vaccination areas should be examined (e.g. monoclonal antibodies, PCR or other appropriate techniques) to ensure that no vaccine-induced rabies has occurred.

7.2.6 Indicators of OVD success

The evaluation of a success of an oral vaccination campaign should be measured in terms of efficacy and safety. Vaccination success should be assessed as a primary indicator of success. Observation data should be collected allowing the evaluation of as many of the following parameters as possible:

- Bait uptake (bait acceptance, bait consumption, dog behaviour);
- Seroconversion in a satisfactory proportion of the target population according to the vaccine under trial. A recognized serological test and a proper statistical evaluation method should be used for this study;
- Dog accessibility to vaccine compared to parenteral vaccination alone (direct observation, biomarker study where direct observation is not possible);
- A reduction in rabies mortality in dogs.

In terms of safety success could be measured by the absence of:

- Vaccine induced mortality in dogs and non-targeted species (typing of virus from diagnostic samples from trial area);
- Human exposure to vaccine (exposure being transdermal or mucosal contact with vaccine).

In addition, it is very important to obtain information on the acceptance of the OVD method by the community (survey before and after bait distribution).

7.2.7 International cooperation

As rabies does not recognize national borders, it is necessary for governments to cooperate at all levels to achieve effective vaccination programmes. Adjacent countries should carefully coordinate their activities along common borders. If field trials reach a country border, local administrative staffs from both countries should coordinate their efforts at the national level. WHO may be particularly helpful in assisting in coordination of rabies vaccination

programmes involving borders between countries.

Oral rabies vaccination generates new epidemiological concerns. For this reason, research should be coordinated by the inclusion of at least two independent WHO-Collaborating Centers in the planning and execution of field trials and post-baiting evaluation phases.

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ANNEX 3 Dog ecology technologies and techniques [28]

1 Surveys

1.1 Preliminary Survey

Qualitative surveys should be carried out in representative communities or neighborhoods to reveal the most influential social and ecological determinants on the dog population size, structure and relationships to humans. Accessibility of dogs to rabies control personnel and volunteers should be the first factor to be examined. In small communities, where the majority of dogs is easily accessible, the ecological questionnaire survey and/or dog marking can be conducted simultaneously with vaccination campaigns. In communities suspected of having dog accessibility problems, and in larger communities requiring operational research for planning, a formal survey should be carried out in randomly selected sample squares.

1.2 The main survey

This should associate (A) a questionnaire survey combined with an observation inventory and (B) visual techniques. Adaptation of the survey methodology to the study area according to the data collected during the preliminary survey and priority given either to questionnaires or visual techniques. The advantages of securing community participation during the implementation of the main survey are given in the chapter on participatory research (iii), see below.

A. Questionnaire survey and observation inventory

This is conducted to quantify and assess through the direct observation inventory the significant parameters that emerge in the preliminary survey. Taking into consideration the population strata and community organization, the following procedure should be followed :

- interview of community leaders and a cross section of community members ;
- obtaining the best census data (man and animals) and available maps of the area concerned ;

Sampling techniques should be adapted to the study area. In villages of under 500 families, all households should be surveyed. In larger communities and cities, samples must be large enough to be statistically reliable. Survey findings from one community should be extrapolated to other communities only with great caution. Smaller surveys in these communities may be used to validate the applicability of earlier inventories.

It should be noted that surveys based on questionnaires alone may lead to a preponderance of data concerning restricted and semi-restricted dogs. This is especially true in rural communities where dogs may regularly move around over large areas. In areas where relatively unrestrained dogs predominate, capture/recapture and dog accessibility studies will help to correct such biases, and may be adaptable to cities where a large proportion of dogs are inaccessible to immediate observation.

B. Capture/recapture techniques and dog accessibility studies (visual techniques).

Where intensive vaccination campaigns are executed or planned, dogs in about 500 neighboring households may be marked at vaccination and re-observed at subsequent intervals so as to assess unrestricted, semi-restricted and restricted dogs. The proportion of marked/unmarked dogs may be used to estimate the dog population size. Where vaccination campaigns are initiated after an initial questionnaire survey, marking/optical recapture methods should be conducted as operational research during the campaign.

It should be kept in mind that any visual technique is only able to provide a valid picture of the real situation at times when most or all dogs are at or near human settlements. In many rural areas, this happens when farmers return from their fields (between 16h00 and 18h00) and/or simultaneously when other family members return (from school or other types of work).

Accessibility of dogs is defined as the percentage of dogs in a given population which can be reached by a person without any special effort. In most cases this person is a member of the household to which the dog belongs. Those dogs which are associated with a household are considered as accessible, as well as dogs which can be approached and touched easily by a stranger (e.g. interviewer). The thoroughness of this inventory can often be augmented when neighboring children are available to guide the interview team to « hidden » dogs.

Simple recording of any visible marks of ownership (e.g. collar) and interrogation of people about the household relation of the dog revealed in a study area in Nepal and Indonesia that over 70% of the animals were associated with one or more households [28]. The remaining animals were approached by the interviewer (a stranger) and their reaction recorded (friendly, fugitive, defensive/aggressive). This brought to 85% the proportion of accessible dogs in all places studied above. It should be noted that recording of about 100 dogs this way can be carried out within a few hours. The consultation group recommends that, at the same time additional information on the sex ratio be noted and possibly be obtained on the extent of supervision (restricted, semi-restricted and whether owner is « nearby »). Puppies should be recorded in respect to litter size. It is also advisable to make inquiries in the neighborhood on the existence of litters because these tend to be hidden and can be missed by the inventory.

Although accessibility can be recorded in this simple manner, actual mobilization through community participation so as to take advantage of the surprisingly high accessibility rates, remains a most important factor. Much more emphasis is needed on this aspect in conjunction with health systems and operational research.

2. Participatory research

Human demographic and social data, dog population and structure, community resources, knowledge, as well as quantitative and qualitative data on all relevant factors can be collected, assessed, applied and evaluated by community residents. The implementation of the techniques mentioned above by community members ensures adequate adaptation to community capacity and relevance of the data collected to the socio-ecology of the community is assumed. In addition, when ecological research is embedded in the context of a

participatory health system control campaign, it increases community awareness in indigenous terms of the rabies problem ; it increases the sense of community ownership and control over the rabies problem and its solution ; and it builds community self-reliance towards the provision of primary health care.

A « community » is here defined as the smallest self-recognized aggregation of households, e.g. neighborhood or village. Participatory rabies research should inventory every household in the community and involve as many individual community members as possible. In the case of a collection of such communities in a town or city, intersectorial collaboration should be instituted in support of each neighborhood programme.

ANNEX 4 Categorization of individual dogs according to restriction and dependency on humans [28]

Comparative studies on the ecology of dogs in different countries have been hampered by the lack of distinct nomenclature and clear definitions of dogs living in a variety of relationships with their human host population. Some of the terms employed in the past include : pet dog, owned dog, community dog, roaming dog, partially protected dog, feral dog, and stray dog. Especially when translated from two or more indigenous frames of reference, these terms become almost meaningless for comparative purposes. The consultative group felt, therefore, that dogs should be categorized on the basis of parameters that are observable, measurable and meaningful under the widest range of socio-ecological conditions met so far by dog ecologists. These two parameters are the level of a dog's dependency on humans and the level of its restriction by humans.

The term stray dog has been particularly troublesome in that (i) very few dogs have been found to have absolutely no association with one or more reference households, and (ii) the term has become synonymous with dogs that must be destroyed. The consultation echoes a proposal that the term no longer be used in an ecological context but rather be reserved for use by animal control officers to apply to dogs not in compliance with local dog ordinances, e.g. not confined/restricted, not on leash, not vaccinated and/or marked/identifiable. Stray dog thus refers to a regulatory situation which generally permits removal of the animal temporarily or permanently from the population.

1 Level of dependence

Dependence describes a dog-man bond based on intentional provision of food, physical shelter, care and any other action to meet the social needs essential for the survival, propagation and well-being of the dog. Thus it excludes unintentional associations of dogs with humans (e.g. feeding in dumping places, shelter in buildings, etc.) as these could also apply to feral dogs in their ecological niche. In this sense, dependency of dogs on humans is a gradient between total and none at all. The consultation proposes the following three categories : full, semi, no dependency.

Full dependency : the dog is given its essential needs intentionally by humans.

Semi-dependency : the dog is given a proportion of its essential needs intentionally by humans.

No dependency : the dog is not given any of its essential needs intentionally by humans.

2 Level of restriction

« Under restriction » should be understood as all physical and biological types of restriction which a human intentionally imposes on a dog. This definition refers not only to movement restriction and confinement of a dog in human's premises but to its supervision outside these premises. A dog which is directly supervised and can be called and thus controlled by a human at any time is considered restricted, e.g. ; dog walking, hunting, herding, leading a

blind person, etc.). Restriction also includes measures controlling the reproduction of dogs, e.g. neutering. In the social context, restriction refers to the control of any contact, association and communication with other dogs and people. Restriction does not include the degree to which care is provided, e.g. food/shelter, since these provisions are included in the notion behind the term « dependence ».

There are three major levels of restriction. In two extreme situations the dog is either totally restricted or not subject to restriction whatsoever. It is understood that some fully restricted dogs are allowed to roam freely for some time each day near their premises to allow urinating and defecating. Such situations in which the dog may or may not be under full supervision and control do not qualify a dog as being partially restricted. The principal characteristic of this group is that the person in charge of the animal intends to have them permanently under control.

Semi-restricted dogs are those under supervision during part of the day either through being used, e.g. hunting, herding, guarding, or for reasons of social association, e.g. affection, resting with the family, joint activities. The restriction may range from 1-23 hours of the day. It is understood that a semi-restricted dog can become, for several days or for longer periods, a fully restricted dog, e.g. a bitch in heat or following delivery, or can become a non-restricted dog, e.g. in periods of a family's absence from their home.

The distinction between non-restricted and semi-restricted is often very difficult, but it is of significance as far as accessibility of dogs for certain disease control measures is concerned, e.g. rabies vaccination. However, the term non-restricted should not be confused with non-accessible since in many societies dogs are allowed to roam freely while being clearly related to reference person(s) and household(s).

3 Matrix assisting in the definition of dog categories

The matrix resulting from the two parameters described above permits a further reduction in the number of categories to possible and essential combinations of levels of care and supervision.

TABLE 1. DOG CATEGORIZATION MATRIX

	1. FULL RESTRICTION	2. SEMI-RESTRICTION	3. NO RESTRICTION
	Dog is physically separated from the rest of the population on a permanent basis	Dog has access to the rest of the population some of the time	Dog has free access to the population at all times
1. FULL DEPENDENCY	RESTRICTED DOG	FAMILY DOG	
The dog is given all of its essential needs intentionally by humans			
2. SEMI-DEPENDENCY		NEIGHBORHOOD DOG	UNRESTRICTED DOG
The dog is given a proportion of its essential needs intentionally by humans			
3. NO DEPENDENCY			FERAL DOG
The dog is given none of its essential needs intentionally by humans			

Since full restriction of dogs excludes them from being semi-dependent or independent, these combinations can be deleted. The same applies to the combination of semi-restricted and fully dependent.

It is relatively easy to define fully restricted dogs which are always fully dependent on humans and those which are not dependent and not restricted which may only unintentionally be given some resources.

After thorough analysis of the other four remaining combinations in the matrix, the consultation concluded that the definition of fully dependent dogs living without any restriction as being more of an academic nature than of any practical value. Certain particular circumstances may force a dog to seek « voluntarily » all its food and shelter from humans who provide this intentionally. However, this situation implies such a close social bond that in most cases supervision and control can be exerted at any time of contact between the dog and

the resource-providing person. It is much more common that dogs living under no restriction are semi-dependent, food and shelter being provided to them both intentionally and unintentionally.