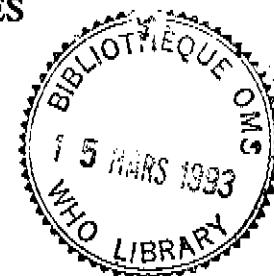




SUGGESTIONS FOR THE DEVELOPMENT OF
A RESEARCH PROJECT FOR THE FIELD EVALUATION
OF SEVERAL VACCINE-BAIT DELIVERY TECHNIQUES
TO VACCINATE DOGS ORALLY AGAINST RABIES

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This document 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 and selecting the delivery system or combination of systems best adapted to 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 in the document may be tested in isolation or combination (eg. door-to-door baiting combined with bait distribution according to the wildlife immunization model - WIM) and could also be evaluated in association with conventional parenteral vaccination strategies (eg. door-to-door or central mobile points parenteral vaccination campaigns associated with bait distribution according to the WIM).

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

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CORRIGENDUM

Page 6, 3.2.1

In place of: "placebo baits (without marker), maps ..."
Read: "placebo baits (without marker) or baits with a serum marker
(only if experiment 3.3 is not carried out), maps ..."

Page 6, 3.2.2, ii.

In place of: "Use baits without marker."
Read: "Use baits without marker if the following experiment (3.3)
is to be carried out, and with marker if experiment 3.3 is
not to be carried out."

Page 8, 3.3.2, i.

In place of: "Distribute baits ...",
Read: "Perform steps 1 to 7 of preceding experiment (3.2) and then
distribute baits ..."

Page 8, 3.3.2, ii.

In place of: "Take blood samples ... roaming the streets."
Read: "Perform steps 8 to 9 of preceding experiment (3.2) and then
take blood samples from a maximum number of dogs roaming the
streets (both marked and unmarked)."

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1. Introduction

In rural and urban areas of many countries of Africa, Asia, and Latin America, canine rabies is a significant public health problem¹. Dog population estimates based on questionnaire surveys and capture-mark-recapture techniques carried out in several countries affected by canine rabies revealed ratios of dogs to human beings ranging from 1:2 to 1:8 in rural regions and the existence of several hundred dogs per square kilometre (sq km) in villages^{2,3,4,5,6,7,8,9,10,11}. In suburban areas ratios of dogs to human beings varied from 1:1 to 1:10 in Mexico City, Mexico¹², from 1:5.8 to 1:11.0 in Guayaquil, Ecuador¹³, and from 1:17 to 1:26 in two study sites in Nigeria¹⁴. Often, the percentage of ownerless dogs remained unknown although in rural and suburban study sites of Turkey and Tunisia it did not exceed 10%^{9,11}.

Mass-vaccination campaigns based on parenteral application of vaccine to dogs were shown to be effective in preventing canine rabies^{15,16,17,18}. Nevertheless, in many countries, oral vaccination might be necessary as a supplementary strategy in order to reach a sufficiently high herd-immunity level.

Several types of baits have been evaluated for the delivery of oral rabies vaccines to dogs^{7,11,19,20,21,22,23}. Some tests for vaccine-bait delivery were carried out in Tunisia. A field trial on a larger scale is now under way in Turkey¹¹. Nevertheless more experimental studies are needed to assess the feasibility of oral vaccination of dogs on a large scale and to develop appropriate bait delivery techniques.

This document describes three main field trials in order to evaluate the vaccination success of several types of oral vaccination strategies using a vaccine-bait system. All experiments should be carried out without vaccine.

2. Requirements

2.1 Bait development and bait selection

For the proposed study, whichever type of bait is selected should correspond to the requirements established by WHO²⁴. To test the preference of the dogs for different baits, WHO recommendations should also be followed²³.

2.2 Markers

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

The use of a topical marker is not compulsory although it may, in some dogs, provide immediate visible information on the contact between the buccal 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.

2.2.1 Systemic marker

It is assumed that a serum marker will be used (sulfadimethoxine [SDM] or iophenoxic acid^{25,26}) but any other kind of systemic marker which does not require post-mortem sampling for detection may also be useful. The vaccine container should be filled with the marker and inserted in the bait. For the second experiment, preferably a marker with a rather long half-life should be used.

2.2.2 Topical marker

Topical markers are Rhodamine B, Methylene Blue etc.^{27,28}. The vaccine container should be filled with the marker.

2.3 Study sites

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

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

2.4 Preliminary experiment: Vaccine-bait acceptance under field conditions

This experiment will provide information about:

- a) bait acceptance;
- b) contact with the "vaccine" (i.e. the systemic or topical marker contained in the vaccine chamber)

2.4.1 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 experiments 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.

2.4.2 Procedure

The experiment should be performed as described below:

- i. Give baits to a minimum of 100 owned dogs selected at random;
- ii. Identify the reference household of each dog (on a map);
- iii. Mark by placing a collar on the dog (or by using a dye), by making a picture, or use two techniques to mark each dog to which a bait is offered;
- iv. Note all important parameters concerning behaviour and bait acceptance (length of the test period; time-span until physical contact with the bait occurs, proportion of bait consumed, whether bait is chewed or not chewed, presence of dye markers on mouth or teeth (if a topical marker is used) etc.) (see recommendations made by WHO²³);
- v. 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 dogs are free-roaming or confined.

The questionnaire should be short and easy to answer;

- vi. On the following day: take a blood sample of each marked dog;
- vii. Check blood samples for marker.

As all dogs offered baits were 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.

3. Testing vaccine-bait delivery systems

3.1 Bait distribution to dog-owners in a central place

This experiment allows an estimation of:

- a) the density of the population of owned dogs (dogs per sq km, ratio of dogs:human beings, dogs per household);
- b) the percentage of households with dogs;
- c) the percentage of dog-owners motivated by the information campaign;
- d) the percentage of owned dogs successfully "vaccinated";
- e) the mean number of baits needed to "vaccinate" one owned dog by this bait-distribution method;
- f) the costs and benefits.

3.1.1 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 (anaesthetic, blow-pipe, dart gun, etc.), questionnaire forms, etc.

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

This experiment should not be carried out in the same village as the preliminary experiment.

3.1.2 Procedure

The experiment should be performed as described below:

- i. Provide advance notice to inhabitants as to the date, time and place of bait distribution. 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, kilometres driven by car, number of announcements per min, costs etc.);

- ii. 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 he has dogs (1 bait per dog). Take into account that dog-owners may probably not declare puppies;
- iii. Note the number of baits distributed, number of dog-owners, number of baits distributed per dog-owner, maybe the dog-owners' names and the total time spent for bait distribution.

Make sure that baits are only distributed to dog-owners who live within the village (boundaries of the village should be clearly defined!). If dog-owners living outside the study area ask for baits, give only placebo baits (without marker);

- iv. On the following day, 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;

- v. 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;

- vi. Check serum samples for marker.

3.2 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 wildlife-immunization model - see section 3.3). It may, however, be carried out independently from the third experiment but the third experiment may not be carried out independently of this one.

This experiment will allow the estimation of:

- a) the number of owned dogs (per sq km, per household, ratio of dogs:human beings);
- b) the number of households with dogs;
- c) the total number of dogs (per sq km, ratio of dogs:human being);
- d) the number of ownerless dogs (per sq km, ratio of dogs:human beings, ratio of owned dogs:ownerless dogs);
- e) the total number of owned dogs "vaccinated" by the door-to-door bait-distribution method;
- f) costs and benefits.

3.2.1 Material

The following should be prepared or made available in advance: placebo baits (without marker), maps, a chronometer, two types of collars (of different colour) and/or two types of dye pens (of different colour), questionnaire forms, material for phlebotomy, dog catching facilities (anaesthetic, 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.

3.2.2 Procedure

The experiment should be performed as described below:

- i. Using the door-to-door method, visit all households, identifying each one on a map;
- ii. Use baits without marker. Give the baits to the dog-owner who should then present one to each of his dogs;
- iii. Note all important parameters concerning behaviour and bait acceptance (see "preliminary experiment" - 2.4.2 step iv);

- iv. Collect baits or parts of baits untouched after a certain time limit (2-5 minutes);
- v. Mark with a collar and/or dye pen etc. every owned dog which has accepted the bait (as in the preliminary experiment);
- vi. By means of a 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 or dye marker used 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;

- vii. Estimate the total dog population by a capture-mark-recapture method (Bailey's direct sampling etc.)^{29,30,31,32,33}. Re-observation should be carried out 3 to 6 times (2 re-observations per day);
- viii. On the following day, 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 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 the 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;

- ix. 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 colour for marking.

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

- x. Check blood samples 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.

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

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

- a) the percentage of owned dogs "vaccinated" by WIM;
- b) the percentage of the total dog population "vaccinated" by WIM;
- c) the percentage of the total dog population "vaccinated" by WIM and the door-to-door bait-distribution method;
- d) costs and benefits.

3.3.1 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 (anaesthetic, blow-pipe, dart gun etc.), binoculars, etc.

The minimum number of persons required for field work is 4.

3.3.2 Procedure

- i. Distribute baits with marker following a preestablished scheme within the study area. Distribute a quantity of baits approximately 3 times the estimated total number of dogs. Bait 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, kilometres driven by car, number of baits distributed, sites where baits were placed, etc);

- ii. Take blood samples from a maximum number of unmarked dogs roaming the streets. Mark every dog from which a blood sample is taken with a collar or dye pen.

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);

- iii. Check blood samples for marker.

4. 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 from these experiments would ultimately allow the selection of one of, or a combination of several vaccination techniques (oral, parenteral). Further studies would then aim at optimizing the selected strategy by modifying the protocol, for example changing the number of baits distributed per square kilometre, 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.

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