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ORIGINAL ENGLISH

# REPORT OF THE FOURTH WHO CONSULTATION ON ORAL IMMUNIZATION OF DOGS AGAINST RABIES

Geneva, 14-15 June 1993

## CORRIGENDUM

| **WARNING** |
|---|
| **THIS CORRIGENDUM RELATES TO LAST YEAR'S REPORT !** |

Page 5. 3.2.1 a) Severe combined immunodeficient (SCID) mice, paragraph 2

Instead of:

"Following intramuscular administration of 30 $\mu l$ of a parenteral strain ..."

Please read:

"Following intramuscular administration of 30 $\mu l$ of a **parental** strain ..."

Page 5. 3.2.1 a) Severe combined immunodeficient (SCID) mice, paragraph 3

Instead of:

"... (in comparison to its parenteral vaccinia virus) ..."

Please read:

"... (in comparison to its **parental** vaccinia virus) ..."

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# REPORT OF THE FIFTH CONSULTATION ON ORAL IMMUNIZATION OF DOGS AGAINST RABIES:

Organized by WHO with the participation of the Office International des Epizooties (OIE).

Geneva, 20-22 June 1994

WORLD HEALTH ORGANIZATION
VETERINARY PUBLIC HEALTH UNIT

# Contents

# 1. INTRODUCTION

The Consultation was opened by Dr F.-X. Meslin, Chief, Veterinary Public Health unit, who welcomed the participants on behalf of Dr H. Nakajima, Director-General of the World Health Organization. Dr Meslin recalled that the last consultation on the subject of oral vaccination of dogs had been held in June 1993 and had led to the establishment of a very comprehensive set of recommendations for the testing of candidate oral vaccines for dogs, as well as for the release of these products into the environment. During the year, new products (i.e. vaccines and baits) or vaccines originally developed for other species were tested in dogs and non-target species under laboratory and field conditions. Also, bait delivery systems were evaluated within the framework of the Programme for research into oral vaccination of dogs in Tunisia. Last but not least, results from dog biology/ecology studies conducted in a number of developing countries became available to further assess the role oral vaccination could play in rabies control and elimination strategies.

The objectives of the fifth consultation, whose membership was extended to a number of potential end-users from Asia, Africa and the Middle East, were therefore to :

a) review the results obtained in the development and testing of vaccines and baits,

b) further assess the safety and feasibility of using the oral vaccination (OV) technique in developing countries for dog rabies elimination and

c) discuss the prerequisites and evaluate the possibilities of launching limited and well-controlled OV projects in selected areas.

Dr A. Wandeler was elected Chairman, Dr G. Thomson, Vice-Chairman and Dr L.E. Robinson, Rapporteur of the Consultation.

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

## 2.1 $SAG_2$

### *2.1.1 In dogs*

An immunogenicity and efficacy study of $SAG_2$ was conducted in five groups of 10 laboratory beagles. Doses of $10^8$ $TCID_{50}$ or $10^9$ $TCID_{50}$ was equally immunogenic when administered with or without bait. The antibody response in groups that received the vaccine directly on the tongue was higher than those vaccinated with baits, but the difference between groups was not statistically significant. No residual infectious virus was detected from swabs collected at 1, 7 and 24 hours after oral administration. By day 180 post vaccination all dogs survived challenge with a street rabies virus, whereas, 8 of 10 control dogs died of rabies.

### *2.1.2 Other carnivores*

The $SAG_2$ oral rabies vaccine was tested in Zimbabwe specifically for the control of rabies in side-striped and black-backed jackals (*Canis adustus and Canis mesomelas respectively*). Efficacy trials have been conducted in both jackal species.

Three of three side-striped jackals and three of three black-backed jackals resisted challenge given six months after an oral dose of $10^{6.5}$ $TCID_{50}$ of vaccine virus. Two of three side-striped jackals and three of three black-backed jackals resisted challenge after an oral dose of $10^{7.5}$ $TCID_{50}$ of vaccine virus. The vaccinated side-striped jackal which succumbed to rabies was a very old animal. A virus was isolated from one pool of saliva suspensions taken on Day 3 from jackals receiving the higher dose ($10^{7.5}$ $TCID_{50}$)

of vaccine. This virus has not yet been typed to confirm whether it was vaccinal virus.

Antibody responses were detected in South Africa in mongooses given high concentrations of virus. Protection was demonstrated in 13/15 mongooses which received a virulent challenge of at least $10^{6.8}$ $TCID_{50}$ by oral instillation. Some of the protected animals had no detectable circulating antibody at the time of challenge.

## 2.2 HAV5RG

Recombinants of the Human Adenovirus type 5 have been evaluated in Canada since 1988. The different constructs have different efficacy, safety and marketing potentials. When given by the oral route, HAV5RG recombinants immunizes foxes, skunks and raccoons (other carnivores have not yet been tested adequately).

Canine adenoviruses immunize dogs by the oral route but no rabies recombinants are available today for experimentation.

## 2.3 SAD B19

Laboratory dogs could be immunized with a SAD B19 vaccine titre of at least 1 x $10^8$ FFU/ml. In two experiments involving 17 dogs collected from the streets in Turkey, all seroconverted after oral instillation of a vaccine titrating 1 x $10^8$ FFU/ml.

A number of owned dogs were vaccinated orally under "simulated field conditions". Dogs which entered the study were permanently restricted but had little contact with their owners. Each dog was first offered a chicken head vaccine bait. If this bait was not accepted the dog was given a Köfte bait (see 4.2.4). Baits contained 1.6 to 1.8 ml of vaccine titrating 1 x 108 FFU/ml. Dogs which took one bait – of whichever type – were bled at the time of bait up-take and, when possible, 3 to 6 weeks later. Blood collection was performed independently of the way the bait was taken (e.g., swallowing of the bait and capsule, perforation of the vaccine capsule). Forty-one dogs were identified for which 2 successive samples were available with one negative on the day the bait was taken. Twenty-five of these dogs (61%) seroconverted (titre equal to or above 1:60). Twenty-three of the 25 dogs (92%) which punctured the vaccine capsule and 2 of the 16 dogs (12.5%) which swallowed the bait seroconverted.

## 2.4 SAD P5/88

As SAD P5/88 is not intended for oral vaccination of dogs, no test on vaccine efficacy in this species was carried out.

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

## 3.1 In non-target species

### *3.1.1 $SAG_2$*

$SAG_2$ vaccine has been administered to several non-target western european species: domestic or wild carnivore and herbivore species, diurnal and nocturnal birds of prey were tested. The oral or intra-muscular administration of the $SAG_2$ vaccine induced neither pathology, nor inter-individual transmission. Rabies virus antigen was not demonstrated either in the brain or in the salivary glands of the animals under test after euthanasia. Several of the species tested so far (*e.g. domestic cat, rat, goat, wild boar and birds of prey*) are common in North African countries where projects for oral vaccination of dogs may be undertaken soon.

Safety trials were conducted in Zimbabwe in many of the locally important non-target bait-consuming species: slender mongooses (*Galerella sanguinea*) and multimammate mice (*Mastomys natalensis*). Mice were each given a dose of 0.1 ml x $10^{9.0}$ $TCID_{50}$. Mongooses were each administered $10^{9.0}$ $TCID_{50}$ of vaccine virus orally. All the non-target animals remained healthy throughout the three-month observation period. Virus was not isolated from saliva swabs 1, 3 and 7 days post-vaccination, nor was virus detected from any brain, salivary gland or tonsil suspensions following the trial period.

In South Africa, no ill effect was observed in groups of either yellow mongooses (*Cynictis penicillata*) or black-backed jackals (*Canis mesomelas*) following the oral instillation of $10^{8.8}$ and $10^{9.3}$ $TCID_{50}$ respectively of $SAG_2$ vaccine. All animals seroconverted. Mouth swabs collected 2, 4, 7 and 9 or 14 days after exposure to $SAG_2$ contained no detectable virus although many 3-5 day-old mice inoculated with suspensions prepared from these swabs died before the end of the 21-day observation period.

### 3.1.2 SAD P5/88

The residual pathogenicity of SAD P5/88 in baboons (*Papio hamadryas*) was examined. Ten baboons were administered orally 2.0 x $10^8$ FFU of the SAD P5/88-strain.

The body weight of all baboons remained within the standard range and all survived the test without signs of illness. At the end of the test all baboons were necropsied and examined for histological alterations in the cerebrum, thalamus, cerebellum, spinal marrow and salivary glands. Neither histological alterations, nor the presence (by FAT) of the rabies virus in the brain and the salivary glands were detected. All but one animal had detectable levels of neutralizing rabies antibodies.

### 3.1.3 HAV5RG

Very high doses (>$10^{10}$ $TCID_{50}$) of HAV5 and HAV5RG recombinants given intranasally to laboratory mice produced an interstitial pneumonia with peribronchiolar and perivascular accumulations of mononuclear cells, necrosis of epithelial cells and macrophages, and massive accumulations of macrophages in the alveoli.

## 3.2 Safety tests in immunocompromised animals

### 3.2.1 $SAG_2$

Viral isolates (mouse brain suspensions) obtained in a previously reported experiment from SCID mice that had succumbed to $SAG_2$ intramuscular or intracerebral inoculation, were inoculated by the intracerebral route into ICR mice. Approximately 15% (14/95) of the inoculated mice succumbed to rabies. Concentrations of virus subsequently recovered were relatively low; all retained the amino acid profile of the $SAG_2$ (glutamate at position 333 of the glycoprotein). Other gene regions were not examined. No $SAG_2$ virus was detected from salivary swabs.

### 3.2.2 V-RG

There was a consistently innocuous outcome following oral administration of high concentrations of the V-RG vaccine to SCID mice. However, a slowly progressive vaccinia infection occurred following parenteral inoculation by a variety of routes including intramuscular, intraperitoneal, intracranial, and intradermal scarification. Time to death and progression of cumulative lesion area was significantly slower with the V-RG virus than with vaccinia.

The only approved pharmacologicical product for vaccinia virus infection in humans is vaccinia immune globulin (VIG). Treatment of SCID mice with VIG at two times the recommended human dose, significantly slowed, but did not stop, disease progression. Similarly, treatment of SCID mice with a new cytosine derivative, hydroxyphosphonylmethoxypropylcytosine (HPMPC 60 mg/kg), slowed disease progression. When combined, HPMPC and VIG prevented the progression of lesions, particularly when treatment was increased from three times a week to every other day and the HPMPC dosage was doubled to 120 mg/kg.

Other immunocompromised host models include domestic cats with Feline Immunodeficiency Virus (FIV) or Feline leukaemia virus (FeLV) infection. Clinical parameters in these animal models (body temperature, weight, white blood cell count, CD4 count, etc.) did not change significantly following oral and intradermal V-RG vaccine administration. Virus isolation was limited to oral swabs taken at one and 24 hours following oral administration. The V-RG intradermal sites were not significantly different between normal and FIV or FeLV infected cats.

### 3.2.3 SAD P5/88 & SAD B19

The modified live rabies viruses SAD P5/88 and SAD B19 were tested in two different strains of immunodeficient mice, nude mice (CD1-nu/nu) and SCID mice (CB17-SCID). All mice were orally and intramuscularly given the same dose of 0.02 ml corresponding to 2.5 x $10^7$ FFU.

In all but one case (due to SAD B19), rabies death was confirmed by FAT. The SAD virus (P5/88 or B19) was detected in the brain, but not in salivary glands, spleen, oesophagus, lungs nor the mucous membrane of the oral cavity of these animals.

TABLE 1 : **Number of rabies deaths in mice tested orally and intramuscularly (i.m.) with two strains of SAD virus.**

| Type of vaccine | SAD P5/88 | | SAD B19 | |
|---|---|---|---|---|
| Route | oral | i.m. | oral | i.m. |
| Nude mice | 0/6 | 3/6 | 2/6 | 6/6 |
| SCID mice | 1/6 | 4/6 | 0/6 | 5/6 |

### 3.2.4 HAV5RG

Adenovirus virus serotype 5 is not usually associated with human disease, but has been implicated in morbidity and mortality in immuno-compromised humans. These cases can be broadly characterized as either primary (eg. SCID) or secondarily acquired, associated with bone marrow transplantation and organ recipients (e.g., liver, kidney, etc.), neoplastic disease and AIDS patients. In these cases, mortality related to viral complications has generally ranged from 33-67%.

# 4. BAIT DEVELOPMENT AND FIELD TESTING

## 4.1 Development of a bait suitable for the oral vaccination of dogs against rabies

The bait system for delivering $SAG_2$ to dogs consists of a solid core containing the vaccine in freeze-dried form. This core has hydrophillic properties and is coated with a protective hydrophobic envelope constituted of substances eliciting food uptake behaviour in dogs. This presentation significantly increased the stability of $SAG_2$ at 20°C compared to the liquid form.

Incorporation of vaccine directly into the core prevents their getting separated by the dog during bait consumption. It also avoids accumulation of non-biodegradable, contaminated vaccine containers in the environment.

Three out of four laboratory beagles accepted such a vaccine-laden bait (DBL-1) immediately after or within hours of presentation. Two out of 4 dogs seroconverted to rabies within 14 days. Results however indicated a negative correlation between the hard texture of the bait and its acceptance, as well as between compactness of the core, time of dissolution and vaccination success.

On the basis of these results a more porous and softened freeze-dried vaccinal core has been designed and incorporated into a new bait prototype (DBL-2). This bait is being tested in laboratory beagles and in Tunisian dogs under field conditions (see 4.2.1.).

## 4.2 Field testing

### 4.2.1 *Tunisia*

a) Bait preference studies in owned dogs

Three experimental artificial dog baits (DBC-1, DBC-2, DBL-1) were tested in owned dogs and compared to chicken heads. Bait acceptance for these artificial baits (DBC-1=36%; DBC-2=37%; DBL-1=52%) was lower than that for the chicken heads.

When 50 baits of each type were distributed to dogs, a coloration of the oropharyngeal mucosa by a topical biomarker (Rhodamine) was detectable in 28% (for DBC-1), 24% (for DBC-2), 52% (for DBL-1) and 56% of the dogs (for chicken heads).

A second trial in owned dogs involved a fourth artificial bait, which contained the serum-biomarker sulphadimethoxine (SDM) (DBL-2 SDM) or did not (DBL-2) and was compared with chicken heads. The acceptance of DBL-2 SDM was 60% compared to 49% for DBL-2 (without SDM) and 59% for chicken heads.

b) Bait acceptance in dogs of unknown ownership status

In Tunisia, the artificial bait DBL-2 was tested in a waste disposal site of Beja in comparison with chicken head baits as reference. None of the baits contained vaccine.

To identify animals which consumed baits, the tracking station method was used. Thirty-one tracking stations were built, each with four sand circles (ø80cm). Two chicken head baits and two artificial baits (DBL-2) were placed in each of 31 tracking stations.

Every three hours the tracking stations were inspected and the baits which had been removed were replaced. Every six hours all baits were replaced. Animals present in the study area were counted every three hours. A total of 200 observations of dogs were made. Three observations of foxes were made.

One hundred and sixty-three chicken head baits were taken (22.03%) out of the total number of 740 baits. In only 17.43% of the cases when chicken head baits were taken could visible dog tracks be observed in the sand circles.

Sixty-four artificial baits were taken (8.73%) out of a total number of 733 baits. About six per cent (5.59%) of available artificial baits (DBL-2) were taken with concomitant observation of dog tracks in the sand circle.

In a precedent study carried out in 1989 in the same area and using the same method, 3.43% of the artificial fish meal baits and 34.29% of the chicken heads were taken with concomitant observation of dog tracks in the sand circle.

c) Systematic delivery of baits to dogs by their owners:

In June 1994, a non-vaccine-laden bait (DBL-2) was tested in two semirural zones in northern Tunisia in order to assess the level of "vaccination success" among the dog population after distribution of baits to dog owners.

SDM was used as a serum marker and indicator of "vaccination success". Baits were given to dog owners at distribution points. On the following days, all households within the study area were surveyed and blood samples were taken from every accessible dog.

A total of 314 baits were given to 178 dog owners. Within the study area, 215 households and 318 dogs were counted during the survey. According to dog owners declarations, 301 dogs were given at least one bait. Ninety-one point seven per cent of these dogs accepted the bait at least partially, and in 84.7% of cases the whole bait was consumed.

Blood samples were taken from 281 dogs. The results of SDM detection are not yet available.

### 4.2.2 Egypt

Dog food manufacturers and suppliers to this industry were contacted to select potential ingredients and enhancers for prototype dog baits. Bait preference tests were undertaken using confined beagles and mixed breed dogs, and also in owned, free-ranging dogs in three towns on the Nile delta region of Egypt. All three groups of dogs showed strong preferences for certain baits or bait coatings. Tallow, egg, cheese, poultry and proprietary products were preferred. Baits made of fish meal and fish oil were less preferred than most other test formulations in all three groups. In Egypt, acceptance of a commercial dog food meal bait coated with 3% beef tallow and a dry cheese product was nearly identical to that of a chicken head.

### 4.2.3 Nepal

A bait preference study was carried out in 1992 which compared the acceptance of four-hand delivered baits presented to owned dogs and dogs of unknown ownership status in Banepa, Nepal. A paired bait test was used to evaluate bait preferences among dogs. Two paraffin baits, one beef and one chicken flavoured, a cylindrical dog biscuit, and locally obtained chicken heads were compared.

Chicken heads were preferred over both types of wax baits. Although more dogs preferred chicken heads than the dog food biscuit bait, the difference was not statistically significant. Chewing time in all four baits averaged between two and three minutes thus potentially permitting oral absorption of a vaccine.

Biomarkers were not used in this study. With chicken heads, a potential vaccination rate of 64% among dogs of unknown ownership status was achieved.

### 4.2.4 Turkey

Initial placebo trials with the "Tübingen fox bait" for vaccine delivery to dogs gave unsatisfactory results. Today a combination of two types of baits is used (i.e. chicken heads – for economic reasons – and "Köfte baits" – made of locally produced minced meat coating the vaccine container). Baits are only given to dogs found in the street. All restricted owned dogs are vaccinated parenterally.

Since March 1994 a field-study on oral rabies vaccination of dogs as a complementary method to parenteral vaccination has been taking place in Istanbul. Out of 1089 dogs which took a bait, one third chose the chicken head bait (offered as first choice) and two thirds chose the Köfte bait. Observation showed that in 306 of the cases (28%) the bait (and vaccine capsule) were swallowed and in 783 cases (72%) the capsule was punctured.

Preliminary results of dog baiting trials in Istanbul, Turkey, showed that the chances of encountering dogs increased at night. At night the proportion of dogs refusing the bait or running away (in most cases) was significantly higher than by day. The average time and distance between two baiting attempts were both significantly lower at night than during the day. In addition to night searches by car, people were interviewed on the street as to where dogs could be found. Using the combined method (by car and on foot) it was possible to significantly decrease the average distance between two successive baiting attempts. However, between this combined method and driving around by night alone, there was no significant difference in the average time between two successive attempts, due to the fact that much time was lost in informing interviewed people in the street about rabies and oral vaccination.

Several different types of vaccine-containers were used to test the influence of their size on the proportion of dogs swallowing the capsule. As could be expected, the bigger the capsule, the lower the number of capsules swallowed. Some dogs chewed the bait before swallowing and thus penetrated the capsule. This was observed by inserting a dye into the capsule.

# 5. DOG BIOLOGY AND ECOLOGY IN RELATION TO RABIES

## 5.1 Philippines

A one-day mass dog vaccination (target of 35 000 dogs) was conducted in a municipality in Metro Manila, Philippines. Urban dog demographic data and vaccination coverage were obtained through the application of pre- and post- vaccination cluster surveys. Household data included human-to-dog ratio, dog introduction/acquisition rate, mortality and whelping rate, dog feeding and biting incident data.

The cost of the programme (excluding manpower) was estimated at US$ 20 400. The cost of vaccinating one dog was US$ 1.00. The breakdown of the costs associated with the major activities of the programme were as follows : Planning and organization 6.8%, health education and information 17.65%, mass vaccination and registration 75.52%.

Person minutes spent per dog per activity were as follows : organization/preparation = 8.43 min, information/education = 7.39 min, training = 8.14 min, vaccination = 13.96 min. Total person minutes spent per dog = 37.92 min.

Evaluation of a mass parenteral vaccination campaign for dogs in Sorsogon Province using the WHO/EPI Cluster survey technique indicated that a 72.5% vaccination coverage was achieved. Additional information collected with household surveys revealed a 3.8:1 humans to dog ratio in this area, 69% of households owned one or more dogs, the median age of dogs in this population was 12 months, most dogs were either given to the household or born in the household, the majority of dogs were considered guard dogs, and most dogs were allowed to roam free during the day and night. A capture-mark-recapture survey (CMR) which was performed concurrently in the same barangays resulted in an estimated 47.4% vaccination coverage. This lower estimate was most likely due to the inadequate marking of vaccinated dogs and the rapid loss of marks prior to evaluation. Other information from the CMR included the dogs activities, habitats, and interaction with humans. Dog density, as calculated by the distance sampling technique incorporated into the CMR survey, was approximately 476 dogs/km2.

### 5.2 Nepal

Preliminary studies on dog ecology were carried out in Kathmandu valley.

House to house surveys indicated that 16% of households owned dogs and that there were 118 dogs residing in 2 wards and the central market area of Banepa, a town of over 13,000 inhabitants. Individual ownership accounted for 73 (62%) of these animals. The majority (72%) of owned dogs were unrestrained and allowed to freely roam in the town. An additional 45 dogs (38%) were found free-ranging and could not be identified as having a specific owner. However 31% of these dogs could be associated with groups of households which provided food and an additional 11% of these animals were cared for by market vendors. All dogs were judged to be approachable for the purpose of oral vaccine administration. Some of the unowned dogs would require capturing and physical restraint to be vaccinated parenterally.

Two thirds of owned and dogs of unknown ownership status were males. Two thirds of dogs of unknown ownership status were considered to be large (>25 Kg), whereas only one third of owned dogs were considered to be large. Rice and dhall are the most commonly fed foods at 97% and 61%, respectively. Food is widely available from village garbage piles and at the market where meat and other food scraps are frequently fed to dogs. In addition, most households feed leftover food to either their own dogs or those in the street.

Only 12% of the owners reported that their dog was properly vaccinated and 17% reported that their dog had ever been vaccinated for rabies. Knowledge of the existence and usefulness of dog rabies vaccine was lacking, although general awareness of rabies was considered to be high. Other reasons for not vaccinating dogs included that the vaccination service was too far away (26%).

Livestock agents suggested a wildlife reservoir for rabies, although no one interviewed reported ever having seen any wildlife species evoking rabies-like signs.

### 5.3 India

It is estimated that the dog population ranges between 25 and 30 million. It is thought that the number of ownerless dogs is higher than those owned. The "stray dogs" (dogs of unknown ownership status) thrive in a community and may be fed by a group of people. These "stray dogs" grow and breed in proliferation. Generally each "stray dog" has its own home-range but moves outside of this range during the breeding season. Stray dog control is directly under the purview of the civic bodies. However, a latest court verdict prohibits indiscriminate killing of dogs and it is suggested that the civic bodies should aim at reducing the dog population by scientific animal birth control measures. Recently, the Animal Welfare Board of India has launched a project on "Animal Birth Control" by mass sterilization and preventive immunization instead of killing the dogs in the major metropolitan cities.

### 5.4 Israel

The mean number of animal rabies cases per year was 20 per year between 1979 and 1990. Since 1991, the mean number of rabies cases per year has increased to 45 per year and in 1993 reached the number of 70 per year.

During the late 1950 s a mass poisoning of jackals reduced the annual number of rabies cases in this species to 0.1 per year and it was only during the period 1992/93 that this number increased to 4-6 per year. Following the compulsory vaccination of dogs together with the elimination of stray dogs, the mean number of dog rabies cases decreased to 6.8 per year during the years 1979-1990. During the same time, the mean number of fox rabies cases increased to 11.8 per year.

The last case of human rabies in Israel occurred in 1960.

In 1993, out of 70 cases, 53% were recorded in foxes while 20% only were recorded in dogs. Only a few cases were reported in jackals. A number of cases were reported in unvaccinated companion animals and livestock (14.2%) throughout the area.

Preliminary oral immunization of wildlife animals using a VRG vaccine in captive foxes and jackals and a few species of local rodents are in progress. Field trials may take place in the near future in a 500 km2 area in the centre of the country.

### 5.5 Morocco

A number of surveys and studies were conducted under the national rabies control plan which started up in Morocco in 1986. The results of those studies are as follows:

- canine ecology:
  - there is an average of approximately two dogs per household in the regions;
  - most of the population (95%) owns at least one dog;
  - 45% of the dogs are over three years of age, 35% are between one and three years of age;
  - the average sex ratio is 3 males : 1 female;
  - 60% of dogs are free at all times and 20% are free either during the day or at night;
  - the general physical condition of the dogs is average;
  - 66% of the dogs guard homes and 31.4% guard flocks;
  - 10% of puppies are kept in the household where they are born.

  Dog population reduction and vaccination have been continuing since 1986. The number of dogs vaccinated were however very low during 1990-1992;

  epidemiological studies show a cyclical rabies pattern between 1978 and 1993. Dogs are the most affected animal species;

- serological monitoring of vaccinations:
  - in 1990, most dogs had no rabies antibodies 30 days after vaccination, and the levels in most animals that did have antibodies generally dropped after the 60th day;
  - in 1993-1994 the antibody level dropped with time;

  the level of public awareness of rabies has improved considerably.

### 5.6 Serengeti Region, Tanzania

A study of dog ecology was initiated because, in addition to public health concerns, canine rabies poses a threat to the conservation of some wild carnivore populations in the region.

Questionnaire surveys were conducted in three study areas adjacent to the Serengeti National Park. Longitudinal studies of a cohort of dogs provided estimates of age-specific mortality, life expectancy and population growth rate.

Blood samples were collected from unvaccinated dogs for rabies serological analysis to determine the existence of rabies seropositivity and to identify risk factors for rabies infection through sero-epidemiological studies.

Frequency distributions of blocking ELISA and RFFIT results suggested the existence of a rabies seropositive population in unvaccinated dogs, however there was only a weak correlation between results obtained from the two analyses. Results of a limited anamnestic response trial in two unvaccinated dogs with detectable antibody (by blocking ELISA) were consistent with prior exposure to rabies virus antigen. Blocking ELISA data revealed significantly more seropositive dogs aged 1-2 years than in other age groups.

The density of the dog population was highest in the Serengeti District (5.17 dogs/km). Preliminary data showed that the population in this district had the highest proportion of young dogs (41.2% less than one year of age), the highest mortality rate in dogs older than three months (47%) and the lowest estimated life expectancy (1.6 years). The Ngorongoro Conservation Area population had the lowest per capita birth rate (41.8%), the lowest death rate (23.5%) and the highest estimated life expectancy (2.5 years).

In two vaccination trials in agropastoralist villages, 68% and 76% of dogs within a 1 km radius of the central-vaccination point were immunized parenterally.

### 5.7 South Africa

Approximately 85% of all cases of canine rabies recorded in South Africa have occurred in KwaZulu Natal. The epidemiology of the disease has been influenced by socio-political, environmental demographic and human factors.

Canine rabies was first confirmed in Natal in 1961, eliminated by 1968 and reappeared in 1976. Since 1976 canine rabies and confirmed human deaths have escalated at an alarming rate on a cyclical basis to reach a peak of 311 confirmed canine cases and 29 confirmed human cases in 1992.

Some recent surveys on dog populations, a burgeoning human population, mass urban migration and socio-political turmoil have underlined the insufficiency of parenteral vaccination alone.

### 5.8 Yemen

The village of Wadi Dhar situated near Sana a comprised approximately 180 households. The total surface of the study area was around 36 ha.

A limited questionnaire survey combined with the marking of owned dogs and an estimation of the number of free-roaming dogs by photographic capture/recapture were carried out.

A total of 30 owned dogs was identified (19 males, 11 females; mean age : 2.6 years, s.d.: 1.85 years). Nine dogs were leashed or shut in, 21 dogs were free-roaming at time of marking. The mean total number of free-roaming (potentially visible) dogs was estimated at 87.5 by direct sampling. When all "invisible" dogs identified during marking were added, the estimated population size was 92 dogs. Using different methods (i.e. Beck's, Jolly-Seber's) it is estimated that the total number of free-roaming dogs in Wadi Dhar ranged between 84 and 122 animals. This corresponds to 230-340 dogs per km$^2$, 0.5-0.7 dogs per household, or 1 dog per 10.7-15.5 inhabitants.

It was shown that :

- The dog density per km$^2$ in a rural area near Sana'a is comparable to that observed in other countries;
- At least 20% to 40% of all dogs in Wadi Dhar were owned.

### 5.9 Zimbabwe

Surveys were initiated to study owner attitudes to dog management and health, structure of and demographic influences on rural dog populations, and social interactions of dog populations.

Two hundred and seventy households were surveyed from three different regions of Zimbabwe. Fifty-seven per cent of households owned dogs. The dogs per capita and per household were 0.2 and 1.3 respectively. The mean age of dogs was 2.5 years. Each bitch produced 0.7 litters per year and 1.7 surviving puppies per year. The main causes of adult deaths were unidentified diseases (38.9% of deaths) and human-related deaths (road accidents, poisoning, etc...) represented 25.2% of deaths.

Dogs are mainly kept as guard dogs (house, crops and livestock). Forty-three per cent of households wanted more dogs than they currently owned.

Dogs were not restricted, except in one case. Most households reported neighbouring dogs scavenging around the house.

During the previous 12 months, 56.9% of the dogs were vaccinated against rabies. Of the dogs that were not vaccinated, 83.9% were born after the last vaccination campaign. About sixty five percent (64.8%) of households questioned knew what rabies was and all households questioned approved of the vaccination campaigns. Almost all households had no objection to the concept of oral vaccination.

## 6. RECOMMENDATIONS

### 6.1 Safety Recommendations

***6.1.1 The following recommendations supersede the first two previously recommended safety tests for modified live virus (MLV) and recombinant live virus (RLV) vaccines listed in WHO/Rab.Res./93.42: Section 6.3.1, a), namely:***

- Safety in puppies

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.

– Vaccine virus excretion in puppies

The possibility of excretion of vaccine virus in the saliva of the animals described above should also be examined. Following immunization, swabs should be taken daily. Recovery of virus in swabs should be consistent temporally and quantitatively with limited viral replication. Any virus recovered should be characterized using monoclonal antibodies or other appropriate procedures. At the termination of the experiment, necropsies should be conducted and relevant major organ systems should be examined for the presence of virus of vaccinal origin.

These should be completed before any oral vaccination field trials in dogs are begun. Any further considerations of safety should be evaluated through placebo studies documenting extent and circumstances of possible human exposure to vaccines.

#### *6.1.2 Techniques for estimating vaccine virus excretion*

As recommended at the 4th WHO Consultation (WHO/Rab.Res./93.42 – section 6.3.4), each reference or regional laboratory testing vaccine virus excretion should determine beforehand the level of sensitivity of virus detection in saliva or faecal samples for each individual test.

Saliva swab samples evaluated by either animal inoculation or cell culture methods currently have been shown to underestimate a known quantity of reference virus by 10 to 100 viral "units" ($MICLD_{50}$, $TCID_{50}$, 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 such details, including swab type (natural or synthetic fibres), 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 quantitation systems such as the double tube system for collection of saliva may be used (see annex 2).

### 6.2 Efficacy

To date, at least two candidate vaccines, the attenuated $SAG_2$ and recombinant V-RG viruses, have met minimum WHO recommendations as regards oral efficacy via a bait against relevant street rabies virus challenge which killed 80% of the controls (see section 3.1.5 on challenge procedures in document WHO/Rab.Res./91.37). National parenteral vaccine efficacy 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 (i.e., bait mixed in food, etc.).

### 6.3 Release of baits into the environment

#### *6.3.1 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.

#### *6.3.2 Types of baits and baiting systems*

Standardized methods for determining dog bait preferences and assessing vaccine bait delivery systems were prepared by WHO (in section 6.1.1 of the report of the 4th Consultation on Oral Immunization of Dogs – document WHO/Rab.Res/93.42 and in Suggestions for the development of a research project for field evaluation of several vaccine bait delivery systems to vaccinate dogs orally against rabies, by H. Matter – document WHO/Rab.Res/93.40) and should be referred to).

The different methods for bait delivery 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").

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

#### 6.3.3 Ecology of target populations

Information on dog ecology and population dynamics is needed in order to develop effective means of bait delivery. Reference should be made to the Report of WHO Consultation on Dog Ecology Studies Related to Rabies Control (document WHO/Rab.Res/88.25), and in particular to its section 3: Improvement of technologies and techniques for the collection of basic information on dog populations.

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 is accessible and available for parenteral 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 parenterally and orally, or in combination.

#### *6.3.4 Further research needs*

Bait development and evaluation should continue for both manufactured and artisanal baits, until data permit formulation of more specific guidelines for implementation of control programmes. Additionally, innovative or novel approaches to the problems should be encouraged.

Limited field trials for the evaluation of factors that will determine whether specific bait and bait delivery systems will succeed or fail should be conducted. These factors include:

■ dog biology:
- intra and inter-species competition for baits;
- availability and attractiveness of alternative food sources;
- dog distribution, densities and demographics;

■ bait distribution:
- extent of training required and availability of personnel for bait distribution;
- bait densities, locations and methods of placement;
- costs of baits and of their distribution;
- socio-cultural acceptance by the local human populations;
- probability of exposure of non-target species to baits;

■ oral vaccine bait efficacy:
- proportion of dogs successfully immunized via baits;
- extent of current rabies surveillance systems and organization of systems which can effectively monitor the success or failure of oral and oral/parenteral vaccination programmes for rabies control/elimination;
- ways to best integrate parenteral and oral vaccination and assess the relative efficacy of mixed parenteral/oral programmes versus an exclusively oral or parenteral approach.
- probabilities of exposure of non-target species to vaccine.

## 6.4 Recommendations for the implementation of oral vaccination projects

### *6.4.1 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, especially in countries and areas where a conscientious application of traditional control measures have yielded less than optimal results from the epidemiological and economical points of view. In dog rabies infected countries where no intensive programmes based on traditional control measures have so far been launched, the initiation of parenteral vaccination campaigns for dogs should allow collection of data on the accessibility of dogs to these control measures and should permit a better assessment of the potential role of the oral vaccination technique.

As oral vaccination programmes should 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 or area may not necessarily be most appropriate for another.

### *6.4.2 Suggested plan of work*

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 subpopulation(s) 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 for implementing oral vaccination projects are:

- to select one or several candidate vaccines. These vaccines should fulfil the requirements for safety and efficacy established by the Fourth and Fifth WHO Consultations on oral vaccination of dogs against rabies;
- to test its (their) safety on major local non-target species competitors for baits. When individuals of the same or a related species have been previously tested for safety with the selected vaccine(s) in a different context, the validity of these results in the local situation should be carefully assessed;
- 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 or subpopulation(s) of dogs which are targeted;
- to evaluate the up-take rates of the chosen bait(s) in the target population;

■ before undertaking any field trial:

- to assess the acceptance of the method by the local people;
- to provide sufficient information to the public so that public support and co-operation is elicited;
- and to study through placebo trials the extent and circumstances of possible human exposure to the vaccine(s);

■ to evaluate in a pilot area:

- the immunization coverage in the target population established by the selected distribution system(s) combined or not with parenteral vaccination campaigns;
- the logistics and costs of vaccine delivery by baits and constraints affecting its efficacy for comparison with those of parenteral vaccine delivery;
- the ability of the local services to monitor human contacts with the bait and the vaccine during the trials and to take appropriate action immediately.

### 6.5 Risk assessment

Risk assessment consists of estimating the probability of occurrence of hazardous situations. This first requires the identification of the possible situation or situations (scenario or scenarios) and secondly, that of the possible chain or chains of events leading to their occurrence(s). The risks (probabilities) are calculated according to the multiplication rules of independent probabilities[1].

In some situations it is not possible to calculate or even estimate the risk of a given event as necessary data may not be available or only fragmentary. In these instances the likelihood of certain events and of the process leading to the occurrence of the hazardous situation may only be expressed as low, medium or high.

The most obvious scenario is the direct exposure of a person through handling of a vaccine bait. 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 immunosuppressed 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, esti-

[1] Paustenbach, D.J. (1989): the risk assessment of environmental and human health hazards: a textbook of case studies. J. Wiley and Sons, New York.

mates should be carried out on the probability of non-target (and especially, human) exposure to the vaccine and possible untoward consequences. An assessment of these risks should take the following elements into consideration:

a) probability of bait contacts:

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

b) probability of exposure to vaccine:

   - for direct exposure : types of vaccine container and vaccine formulation (liquid/lyophilized) may play a role through vaccine spillage, rejected vaccine containers or bait fragments..., etc;
   - for indirect exposure (via a bait consuming animal), the species involved, its age and immunological status may be important.

   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.

c) probability of disease occurrence:

   - type of vaccine and type of disease;
   - level and duration of excretion of virus in target and non-target animal species;
   - undetected/unreported exposure;
   - availability/unavailability of specific treatment;
   - prevalence of immunodeficiency syndromes in human and animal populations.

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. 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 the magnitude/nature of contacts between baits, vaccine containers, vaccines and non-target species, especially humans.

It is also recommended that the group during its next meeting prepares guidelines for the assessment of certain risks related to oral vaccination of dogs, on the basis of a draft to be compiled beforehand by the VPH unit. ■

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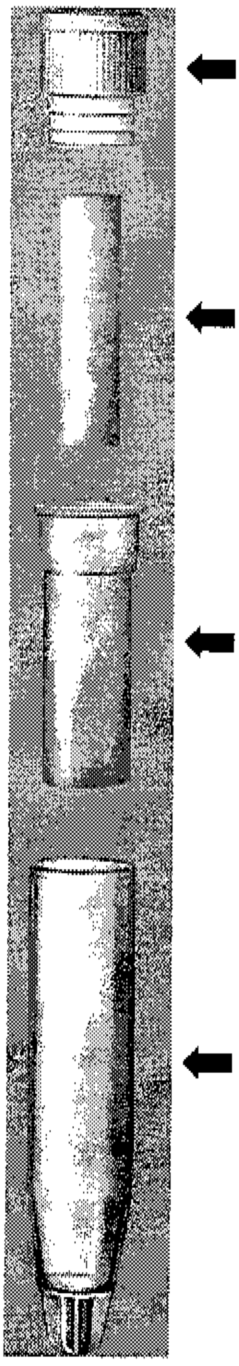
# ANNEX 2

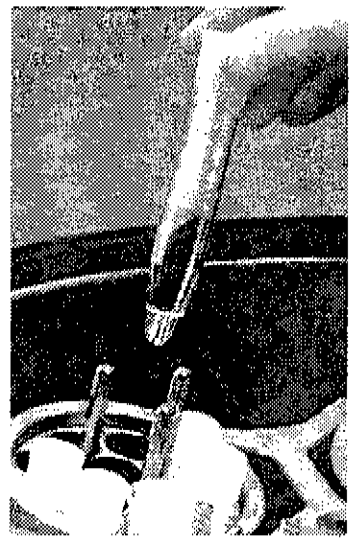
## *SALIVA QUANTITATION SYSTEM*

Saliva is collected through chewing a cotton wool cylinder held in forceps by the animal or swabbing of the oral cavity. The imbibed cylinder is placed in an inner tube (holed at the bottom and closed by a screw-cap) and then is slotted into an outer tube (picture 1). Saliva is extracted by centrifugation (picture 2) and collects in the lower part of the outer tube (picture 3). The system is convenient, hygienic and facilitates transportation and storage.

**Picture 1**




**Picture 2**



**Picture 3**