Oral Immunization of Dogs against Rabies: Report of the Sixth WHO Consultation

with the participation of the Office International des Epizooties (OIE)

WORLD HEALTH ORGANIZATION

Zoonotic Diseases
Division of Emerging and other Communicable Diseases Surveillance and Control (EMC)
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1. INTRODUCTION

During the last consultation on this subject the group expressed the opinion that time was ripe, should final safety and placebo bait delivery tests be successfully performed, for initiating small-scale efficacy trials in selected areas. No such controlled trials have been started at the date of this meeting as delays occurred in the implementation of the safety tests required.

Data have become available, however, on important aspects of the OVD concept in particular on bait (non-vaccine loaded) delivery under field conditions. This 6th consultation should be seen as the last of a series which started in 1988, the aim of which was to define prerequisites and requirements for the voluntary release of live vaccines for the oral vaccination of dogs in developing countries. This consultation should, therefore, in addition to reviewing the results of recent research, evaluate the work achieved by the series of meetings on OVD organized by VPH.

It is foreseen that a new series may begin as soon as the first results of efficacy field trials become available.

Dr F.-X. Meslin welcomed the participants on behalf of the Director-General of the WHO, wished them an excellent stay in Geneva, and very fruitful discussions. Dr B. Perry was elected Chairman and A. Kappeler was nominated rapporteur.

2. DATA ACCRUED RESULTS OF RECENT RESEARCH

2.1 LIVE ATTENUATED VACCINE

2.1.1 Safety of SAG₂

- in laboratory dogs:

SAG₂ vaccine was studied in 12 beagles. Six dogs were inoculated orally with 1 ml SAG₂ containing $10^{8.5}$ TCID₅₀/ml. Another group of six dogs was inoculated with similar amounts of virus into the brachial nerve plexus and surrounding tissues. Daily observations did not reveal any clinical signs. No virus could be detected in saliva swabs collected 1.7 and 24 hours after vaccination. All animals seroconverted by day 15, and the dogs inoculated in the brachial plexus even earlier i.e. by day 7. One dog from each group was euthanized on days 3, 7, 10, 15, 25 and 35. Tissue collected at necropsy were tested for infectious virus by tissue culture and suckling mouse inoculation, but no viable virus was detected.

- in South-African puppies:
SAG₂ at a concentration of approximately $10^9 \text{TCID}_{50}$ (10$^{7.2} \text{MICLD}_{50}$) was administered orally and by the intramuscular route to 2 groups of 10 indigenous puppies each aged from 7 to 10 weeks. None of the animals developed clinical signs which could have been related to vaccine administration during the first 90 days of the 120 days observation period. Rabies virus was isolated from 7 out of 10 and 8 out of 10 of the saliva swabs taken 1 hour after oral or intramuscular vaccination but not at any later occasion. The virus was characterized as SAG₂. Serum and post-mortem FITC analysis are pending.

-in indigenous dogs and cats in Tunisia:

Safety trials on indigenous dogs (8 adults, 3 juveniles) and 10 puppies (<10 weeks old) received orally $10^9$ TCID$_{50}$ of SAG₂. No vaccine induced disease or death was observed in any of the animals throughout the observation period (90 to 180 days). However 6 of the 10 puppies died between day 2 and 24 after vaccine administration. No viral antigen or virus was detected in the brain smears, and no viral antigen in the salivary glands or maxillary and parotideal lymphnodes of any of the animals which had died or that were euthanized at the end of the experiment. Serum and saliva results are pending. A similar trial involving adult cats and kittens is in progress.

- in non-target species in Zimbabwe:

- Honey badgers (Mellivora capensis)

Six honey badgers were administered 1ml x $10^9$ median tissue culture infectious doses (TCID$_{50}$) of SAG₂ virus orally under light ketamine/xylazine anaesthesia. They were observed for 92 days after administration, no ill effects occurred. Salivary swabs were taken 1, 3 and 7 days after vaccine administration and tested for rabies by intracerebral inoculation into suckling mice. No virus was isolated. Only four of the six honey badgers seroconverted.

- Pied crows (Corvus albus)

Six crows were tested by the administration orally of 1ml x $10^9$ TCID$_{50}$. No anaesthesia was used. No ill effects of the vaccine were noted during 94 days of observation. No salivary swabs were taken. None of the crows seroconverted.

- Gerbils (Tatera leucogaster)

Twenty-six gerbils were given 0.1ml x $10^9$ TCID$_{50}$ by the oral route without the use of anaesthesia. None died of vaccine-induced rabies during the 94 day observation period. One died 42 days after vaccination: its brain was negative for rabies using the FAT.
2.1.2 Safety of SAD B19 in non-target species

- in cats

Two groups of 5 and 7 cats, caught in Ankara, were vaccinated orally with 1.5 ml of SAD B19 at 2 different concentrations: 3-5 x 10^7 FFU/ml for the first group and 2.2 x 10^6 FFU/ml for the second. The first group was observed for 84 days, and the second group for 183 days. None of the animals showed any clinical symptoms of rabies during the observation period or died from rabies (FAT negative)

- in rodents

A total of 81 individuals from 5 different rodent species (Mus musculus, Rattus norvegicus, Microtus epiroticus, Apodemus sylvaticus, Apodemus agrarius) were captured locally and inoculated orally with SAD B19. A total of five rodents died from rabies three of which were anaesthetized to facilitate vaccine administration. Transmission of vaccine from inoculated rodents to control animals of the same species was not observed. Suckling mice borne from vaccinated mother did not show any sign of sickness or died from rabies.

2.1.3 Dog immunization with a bait (DBL-2) containing freeze-dried SAG2

Two groups of four laboratory beagles (5-6 months of age) were vaccinated with a single dog bait (DBL-2) containing respectively 10^8 and 10^9 TCID<sub>50</sub> of the freeze-dried rabies vaccine strain SAG2. Three out of four animals of either group survived a challenge with a canine street virus strain (MA-85P1) carried out 28 days post-vaccination, that killed two out of two unvaccinated control animals. None of the vaccinated animals developed a detectable titre of rabies virus neutralizing antibodies, and only one animal exhibited a low antibody response towards the rabies virus nucleoprotein

2.1.4 Immunogenicity of SAD B19 in dogs in Turkey

- in the laboratory

Twenty free-roaming dogs caught in Ankara were vaccinated orally (by directly instillation of the vaccine into the mouth cavity). Ten animals were vaccinated with 1.5 ml containing 10^8 FFU/ml and 10 other dogs with 3 ml containing 10^7 FFU/ml. Blood samples were collected on a regular basis. Neutralizing antibodies were detected by RFFIT in all dogs having received 1,5 ml of vaccine after 14 days against only 4 of the 10 dogs which had received 3ml of vaccine. These titers dropped rapidly as after 93 days only 1 out of the 10 dogs had detectable leve of VNA.

- in the field

Approximately 3.6 ml of SAD B19 containing 4.2 x 10^7 FFU/ml were given to turkish dogs kept under field conditions in the Anatolian part of Istanbul. Blood samples were taken
immediately and, on average 22 days after vaccination. Ninety percent of the dogs (89/99) showed a response above 0.5 IU per ml. No sex- and age-dependent differences were observed in the seroconversion titers. No significant difference in titers was found between owned and ownerless dogs. Five months after vaccination 45 dogs were relocated and a third blood sample was collected: 13 (28.9%) of the dogs had no seroneutralizing antibody-titer, the rest of the dogs (71.1%) had a titer of 1.0 IU or more. In addition no significant difference in seroconversion titers was observed when comparing vaccine delivery via bait (Köfte-bait) and vaccine instillation in the mouth cavity.

2.1.5 Bait delivery

- Distribution of placebo DBL-2 baits in Tunisia.

- according to the WIM:

The study took place in a semi-rural area west of Tunis, El Bessatine, with 2400 inhabitants and 337 dogs, of which 35% were roaming free during the day according to owners. The baits distributed were DBL-2 without vaccine containing a biomarker SDM.

About 1200 baits were distributed according to the wildlife immunization model "WIM", along road sides and around the village at a density of 4.7 baits/dog. Twenty hours later, 40% of the baits had disappeared. SDM results of 293 blood samples collected revealed that 23% of owned dogs had consumed the bait from which 7.5% were considered inaccessible to parenteral vaccination. Forty percent of dogs with unknown ownership status from which serum was collected were SDM-positive.

- through dog owners

Two limited field trials on bait delivery through dog owners were carried out in a semi-rural zone of Tunisia. In the first trial, 314 baits were distributed to dog owners at four bait delivery points. Baits were without vaccine but contained SDM as a biomarker. Bait acceptance was evaluated by a questionnaire survey and SDM was detected in the serum of about 85% owned dogs. According to the indications of the dog owners, bait were given to 90.3% of all owned dogs in the study area. Of these 96.5% accepted the bait at least partially. This corresponds to an overall bait-uptake rate of 87.2%.

The SDM test gave a positive result in 93% of the dogs from which a serum sample was taken. Assuming a zero SDM prevalence in dog sera from untested dogs which did not have access to a bait, and a positivity rate of the untested dogs with bait access equivalent to the one of tested dog with access (95.4%), the overall prevalence of SDM in the dog population was estimated to be 86.2%. In conclusion between 85 and 90% of the owned dog population in the catchment areas actually consumed a bait, at least partially.

In the second trial, 295 baits (containing Rhodamine B instead of vaccine) were given to owned dogs following the door-to-door bait delivery model. Bait acceptance was evaluated by direct observation. Sixty-five percent of juvenile and adult owned dogs accepted the bait within the average time of 46 sec. until contact with topical marker. This
corresponds to an overall vaccination rate of 51.3% (juvenile and adult dogs).

The vaccination rate in total owned dogs was higher in the first trial, but there were some differences between the two trials such as region and essential time bait exposure (18 hours in first trial and 10 minutes in door-to-door bait distribution).

- studies on a baiting procedure for jackals (using chicken heads, commercial blisters and rhodamine B)

Ten side-striped jackals and five black-backed jackals were used for this trial.

Chicken head baits were prepared by inserting a rhodamine B filled Virbac vaccine blister under the skin of each chicken head and stapling it into place to prevent it falling out. Each jackal was offered one bait at a time. The baits were checked regularly and when consumed the jackals were immediately anaesthetized with 100mg ketamine, after which the oral cavity, including the pharynx, was checked for staining. External body staining, and stain spillage on the floor of the pen were also noted. The fate of the capsule was recorded.

Of the 30 attempts to bait the jackals, two resulted in subsequent vomiting and three did not consume their baits during the day of the trial.

Most of the baits were consumed within 10 minutes of being offered, in a few cases they were consumed by the jackal immediately on being offered. In a few cases the jackals moved the baits around before consuming them, sometimes puncturing the blisters in the process causing leakage of stain.

Generally the staining was most prominent on the dorsal and lateral aspects of the tongue, the hard and soft palates and the inner gingival surface. The pharynx was generally not as heavily stained, although when there was heavy staining of the tongue and palate, there was prominent staining of the pharynx. Staining of the outer gingival surfaces and the inner cheek membrane was always less heavy and more irregular than with the other oral structures.

Some amount of stain was usually wasted by spillage onto the floor or onto the external skin, usually the face and the front legs and paws. However, there did not appear to be a significant inverse relationship between oral staining and stain spillage, with some jackals causing considerable spillage, but also having heavy oral staining.

- köfte bait up-take by dogs and competitor species in Turkey

In Turkey 95% of the dogs offered a Köfte-bait took it without hesitation. The ‘vaccination-rate’ with the Köfte-bait is unfortunately much lower than the bait acceptance-rate, as the vaccine-container is often swallowed without chewing. An increase in vaccine-container size reduced the swallowing-rate, but even the largest vaccine-container tested was swallowed by 32% of the dogs when baits were offered directly. A new capsule is under
development. The high swallowing-rate of the vaccine containers may partly result from the unusual conditions surrounding bait delivery. The swallowing-rate was significantly lower when baits were placed at given sites where the dogs found them by themselves.

Placebo köfte-baits were placed at selected sites. The bait disappearance-rate was very high in urban areas: on average 50% of the baits were taken after 275 minutes. During the night the proportion of baits taken by dogs was significantly higher than during day-time. At night competition from free-roaming owned dogs was lower than during the day, thereby increasing the chances for ownerless dogs to encounter a bait. During day time crows located 30.1% of the baits. During the night cats were the major bait-competitors taking up to 27.3% of the baits.

### 2.1.6 SAG₂ thermostability in Zimbabwe

Two enclosures, approximately 9 m², were built on the laboratory field station 30km north of Harare to incorporate baits while preventing interference by wild animals. One was situated under the shade of a tree, the other was in open sunshine.

SAG₂ vaccine with a titre over 9.0 log₁₀ TCID₅₀ was diluted 1 in 10 with RPMI HEPES-buffered cell culture media. A volume of 2ml of the vaccine was then dispensed under sterile conditions into 100 Berne Type I blisters. The blisters were heat sealed, inserted under the skin of chicken heads and stapled to the skin.

Four treatment groups were set out as follows:

- **Group A**: 25 heads set in full sunshine, no cover;
- **Group B**: 25 heads set in full sunshine under dense dry grass cover;
- **Group C**: 25 heads set under shade, but with no cover;
- **Group D**: 25 heads set under shade under dense dry grass cover.

The baits were set at 0800 hours. One head from each group was taken at the time of setting the baits (T₀) and was transported on ice back to the laboratory. Thereafter, four baits were taken from each treatment at each of the following time periods: 6, 12, 24, 48, 72 and 96 hours. They were transported back to the laboratory where the blisters were removed from the heads, cleaned, and the vaccine removed under sterile conditions using a syringe and needle. The vaccine from each blister was placed into 1.8ml cryotubes and was stored down into liquid nitrogen.

Vaccine titrations were done at the end of the trial, using pooled samples.

Two trials were carried out in July 1994 and October 1994.
The vaccine titre declined markedly when the baits were placed in direct sunshine, particularly during the October trial, when the titre declined to nil after 24 hours. The decline was less marked when placed in the shade, with no cover. There was no apparent difference between the two groups of baits placed under cover. In both cases the decline of titre was 1 log<sub>10</sub> in three days.

### 2.1.7 Oral efficacy of SAG<sub>2</sub> in Coyotes

Wild-caught coyotes (*Canis latrans*) were divided into 3 groups of 5 animals each: 5 controls and 10 vaccinates, which received either 10<sup>8.0</sup> TCID<sub>50</sub>/m. of SAG<sub>2</sub> virus. Animals were bled weekly for the induction of rabies VNA by the RFFIT. No control coyotes developed VNA, while 5/5 and 4/5 vaccinated animals did produce detectable VNA in the 108,00 TCID<sub>50</sub>/ml groups, respectively. Vaccinated coyotes were protected against a virulent street virus challenge of coyote origin, with the exception of the single animal that did not produce VNA. No suggestion of illness was observed in the vaccinated coyotes as a result of exposure to SAG<sub>2</sub> virus.

### 2.2 Live recombinant vaccines

#### 2.2.1 HAVR in Wildlife

Four genetically engineered HAV5 rabies-glycoprotein recombinant vaccines were used for oral immunization experiments in order to evaluate their potential as wildlife vaccines. All constructs were made by Dr. L. Prevac and colleagues at the Department of Biology, McMaster University, Hamilton, Ontario. In Ad5-RG1, Ad5-RG4 and Ad5-RG1.3, the rabies glycoprotein gene is inserted into the deleted E3 region of the HAV5 genome. With Ad5-RG4 and Ad5-RG1.3 the rabies glycoprotein expression in infected tissue culture is better than with Ad5-RG1. Ad5-*E1-RG* is a recombinant with the rabies glycoprotein gene inserted into the deleted E1 region of the genome. This recombinant is not capable of replicating in hosts other than 293-cells. In all four constructs the insert is a c-DNA of the ERA strain rabies glycoprotein gene, which may be protected by patents. Ad5-RG1 when given by the oral route induced the production of rabies neutralizing antibodies in foxes, skunks and raccoons. Seroconverting foxes and skunks resisted challenge with street rabies virus.

The 50% effective dose of Ad5-RG1 in skunks is between 10<sup>5.5</sup> abd 10<sup>6.5</sup> TCID<sub>50</sub> when the vaccine is given directly into the oral cavity; 10<sup>8</sup> usually immunizes all animals. The inclusion of Ad5-RG1 into baits (OMNR blister, sponge, Dupont) greatly reduces its efficacy. Pre-existing immunity to HAV5 interferes with oral immunization with the Ad5-RG1 recombinant. Skunks do not respond very well to Ad5-RG1 when the vaccine is deposited directly into the small intestine. This observation indicates that a bait releasing the vaccine into the oral cavity (rather than into the digestive tract) probably will be essential.
Ad5-RG4 and Ad5-RG1.3 were compared with Ad5-RG1 and with Ad5-*E1-RG in several oral immunization trials in skunks. It was found that lower virus titres of Ad5-RG4 and Ad5-RG1.3 were required than with Ad5-RG1 or Ad5-*E1-RG for inducing measurable immune responses. Ad5-*E1-RG efficacy in skunks is similar to Ad5-RG1. Ad5-RG1.3 is presently chosen as a possible candidate for field testing in Canada. The 50% effective dose leading to seroconversion in raccoons is approximately 10⁶TCID₅₀. A 100-fold higher dose is required to immunize 50% of raccoons if the vaccine is included in blister pack baits.

2.2.2 Genetic stability of V-RG in vivo

Upon request of the Canadian regulatory authorities the pathogenicity and genetic stability of V-RG has been assessed in vivo. Scid- and Nude mice were inoculated with 10⁶.⁵ TCID₅₀ by 3 different routes. As in previous studies (Hanlon et al.) it was confirmed that V-RG, upon intracerebral inoculation or scarification, caused lesions in most of the animals and some mortality in both strains, but no earlier than 31 days p.i. After oral inoculation no mortality was observed and only a small proportion of scid-mice showed minor lesions and allowed for isolation of the virus.

Surviving animals from each route and each strain were sacrificed and tissue samples collected at day 5, 10, 20, 40 and 80 after inoculation. Tissue suspensions were inoculated into Vero cells and positive isolates were tested for the presence of rabies glycoprotein insert using PCR. A product encompassing both the thymidine kinase gene and the rabies glycoprotein inset could be amplified from all isolates and no indication was found that the inset had been partially or completely deleted in any of the more than 600 virus isolates analyzed. Restriction enzyme analysis (REA) and limited sequencing revealed some variation in the length of the poly-A tail located downstream of the coding region of the rabies glycoprotein insert. No other deviations became obvious using REA.

Cell culture passages of a third of all isolates contained a usually low proportion of plaques that were not stained with two out of three different anti-rabies glycoprotein mAbs. Limited sequencing of 14 of these isolates revealed 1 or 2 additional ‘G’s after a homopolymer of 6 ‘G’s in the coding sequence of the rabies glycoprotein gene. The resulting frameshift leads to early termination of translation after less than 400 amino acids. There were indications that these mutants had been introduced in a very low proportion when the mice were infected, as plaques with the same staining characteristics were observed in controls using the inoculum virus. The cell culture passage of a single isolate contained plaques that were not stained with any of the three anti-rabies glycoprotein mAbs. Sequencing of the isolate revealed an additional ‘C’ after a homopolymer of 4 ‘C’s in the coding sequence of the rabies glycoprotein gene. Again, the resulting frameshift leads to early termination of translation.

Although the methods applied are unable to detect minor deletions/insertions, except for those occurring at restriction sites or those leading to changes in the epitopes recognized by the mAbs used in screening tests, it can be concluded that mutations
rendering the rabies glycoprotein gene insert and/or regulatory sequences non-functional occur at a low frequency.

### 2.2.3 Potential shedding among dogs vaccinated orally with V-RG

To ascertain the likelihood of viral shedding in domestic dogs which may contact vaccine, 16 adult beagles were administered 1.0 ml of a vaccinia-rabies glycoprotein (V-RG) recombinant virus. Six of the dogs were naive to rabies vaccine and 10 had been previously vaccinated by the parenteral route with rabies vaccine. Eight randomly-distributed, non sedated dogs were administered $10^9$ TCID$_{50}$/ml of the V-RG virus, while 8 others received $10^{8.5}$ TCID$_{50}$/ml. Dogs were given vaccine by manually opening the mouth and distributing virus into the oral/pharyngeal cavity. A sterile, polyester fibre-tipped applicator was used to obtain a sample from the buccal cavity and rectum of each animal. Dogs were swabbed prior to, and at 1, 24, 48, 72, 96 and 168 hours after oral V-RG virus administration. Samples were stored frozen in transport media at -70°C until PCR analysis. Dogs were also bled on day 0 and weekly thereafter until day 30 for the determination of rabies virus-neutralizing antibodies (VNA) by the RFFIT procedure. No virus was detected from any faecal swab sample; evidence for residual input VR-RG virus was found in oral swabs at 1 hour in 56% of the dogs. Rabies VNA were not detected among the naive animals. Previously vaccinated dogs had rabies VNA present on day 0; 9/10 demonstrated an anamnestic response. All animals remained healthy throughout the study.

### 2.3 Further studies on inactivated vaccine

Two dogs were administered purified RNP vaccine (ERA virus) at 2 doses and were challenged with a coyote/dog street virus approximately 4 months later; both dogs succumbed. No inactivated vaccines have suggested oral efficacy in dog trials to date, unlike other carnivores.

### 3. Dog population studies

#### 3.1 Estimating recapture probabilities and the proportion of free-roaming dogs by a capture-mark-recapture model

Several strategies of oral and parenteral vaccine application which target different subpopulations of dogs are now available. Appropriate experimental designs which provide precise estimates of dog population sizes are needed in order to assess the potential use of these new vaccination strategies.

Data of a limited field trial on bait delivery in El Bessatine (Tunisia) was used in a Bayesian model designed for making inferences on model parameters: the size of different subpopulations of dogs and their recapture probabilities. The proportion of free-roaming dogs that either accepted or refused a bait during a house-to-house bait-delivery campaign was of
A house-to-house survey including all households in the study area provided prior estimates about the model parameters. Prior distributions were updated by the recapture data leading to posterior distributions yielding all the relevant information such as means, standard deviations, and credibility intervals. The distribution of the total number of free-roaming dogs was simulated from the corresponding distributions of the three subpopulations of bait accepting dogs, bait refusing dogs, and dogs to which no bait was presented.

Of all owned dogs in El Bessatine, 33.1% (95% confidence interval 29.4%-36.9%) were estimated in our model to be free-roaming during daytime. This corresponded well with the figure derived from a questionnaire survey including all dog owners in El Bessatine (37.5%). However, it was much less than the unadjusted average proportion of free-roaming dogs in the reobservation samples (75.4%).

Of the dogs which refused the bait during the house-to-house bait delivery campaign, 44.9% (95% C.I. 39.0%-52.4%) were free-roaming. This was significantly more than the free-roaming proportion of bait accepting dogs (21.5%; 95% C.I. 17.2%-26.0%). These figures are important in the evaluation of the efficacy of different bait delivery models.

The Bayesian approach has two advantages. It allows for (1) incorporating prior knowledge on all parameters in a flexible way, and (2) simultaneously estimating subpopulation sizes and recapture probabilities, which is an essentially undefined problem in the classical approach.

3.2 CHARACTERISTICS OF THE DOG POPULATION IN ISTANBUL

Out of 10137 households visited in seven areas of Istanbul only 5.2% owned one or more dogs. The highest percentage of households with dog(s) was found in rural areas e.g. Hüseyinliköy (45.7%) and Cavusbasi (19.1%). The lowest percentages of households with dog(s) were found in areas with high apartment buildings with few (public) open areas e.g. Erenköy (4.3%) and G. Osmanpasa (0.1%). With an estimated 12 million people in Istanbul, an average number of people per household of about 4.4, the total number of owned dogs was estimated to be around 150.000. However, the number of ownerless dogs remains unknown in the city. The average owned dog to household ratio in Istanbul was 1:18 and 41% of the owned dogs were 1 year old or less. The sex ratio of owned dogs was 6.8 males per female dog. In the neighbourhood of Kavacik the overall dog population size was estimated by a mark-recapture-survey giving an estimated density of 166 dogs per km$^2$. Approximately 30% of these dogs were considered ownerless. The estimated annual turnover of the owned dog population in Kavacik was around 50%. In Tokatköy and Hüseyinliköy, respectively 86.3% and 9.1% of the dogs were always restricted. Most owners (81.7%) keep dogs to guard the house.

The average vaccination coverage of the owned dog population in areas investigated was 31.9%. A vaccination coverage of 70% and more was achieved only in the high income urban area of Kanlica and the rural village of Hüseyinliköy. The coverage in low-income urban areas averaged only 25.4%. Large spatial differences in the parenteral vaccination
coverage exist.

On the basis of data collected through a questionnaire-form from registered private veterinary clinics it was estimated that about 41 000 dogs were vaccinated each year in the city. In 1994 the Provincial Veterinary Office in Istanbul distributed 6000 of vaccine. Therefore a total of 47000 dogs were vaccinated parenterally against rabies in 1994 with an estimated owned dog population of 150,000 animals.

4. **Evaluation of the Series of WHO Consultations on Oral Vaccination of Dogs**

The group assessed the value of previous recommendations made during the series of WHO consultation on this subject regarding especially safety, efficacy and bait delivery as well as their level of implementation. Regarding safety and efficacy, the group felt that the current recommendations are in general sufficient and specific enough. It was also considered that these recommendations have been met at least partially by most of the candidate vaccine strains. However, the group identified only a small number of vaccine candidates which had fulfilled most requirements, and no vaccine which met them all. It was felt that recommendations related to bait delivery were incomplete. The exploration of novel approaches must be encouraged. The importance of operational research and proper data collection was pointed out. With the exception of one country where a number of delivery trials using placebo baits have been conducted, most of these recommendations have not been implemented. The economics of bait delivery should be given priority in future.

5. **Final Conclusions and Recommendations**

5.1 **Safety Requirements**

Current recommendations for safety testing are summarized in section 6.3 of the report of the 4th WHO Consultation on Oral Immunization of Dogs against Rabies (document WHO/Rab.Res/93.42) and superseding modifications referring to safety tests in puppies and the collection of saliva samples or faecal swabs are given in section 6.1 of the report of the 5th WHO Consultation on the above subject (document WHO/Rab.Res/94.45). The group evaluated, which of the recommendations have been met in full or partially for existing candidate vaccines, based on existing written documents and table 1 given in Annex 2. While the majority of the recommendations concerning safety have been met by many of the candidate vaccines, special emphasis needs to be put on the following issues:

- Safety for target animals

! Assessing safety (and efficacy) in indigenous dogs (influence of health status), orally and parenterally.
safety testing in dogs under the age of 10 weeks, orally and intramuscularly.

- Safety for non-target

Local wild and domestic animal species that may take baits should also be given a dose of vaccine orally equivalent to 10 times the field concentration in a volume adapted to body weight. 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.

- Virus excretion

Residual virus and excretion in puppies and in older dogs

- Surveillance of human exposure and risk management evaluation

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.

There is no evidence of salivary excretion of oral rabies vaccines (V-RG, SAG$_2$), 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 48 hours.

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.

5.2 Efficacy of Oral Vaccines

Results of Immunogenicity and efficacy studies carried out with various vaccine candidates are summarized in tables 2 to 5 in Annex 2.

For bait preference studies recommendations in section 6.1.1. of the report of the 4th Consultation on Oral Immunization of Dogs against Rabies should be kept (document WHO/Rab.Res/93.42). 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 at best contact with the vaccine.

In previous consultations recommendations have been made to extend bait delivery tests to as many different areas as possible. When doing this, consideration should be
given to social and cultural human factors, human density, and dog population structure, dynamics, and feeding patterns.

Field trials, including bait acceptance and bait delivery, have been conducted in Tunisia and Turkey. However no economical study was carried according to the recommendations set out in section 3.3.5 of the report of the Second Consultation on Oral Immunization of Dogs against Rabies (document WHO/Rab.Res/91.37). Cost-effectiveness of the different vaccine delivery strategies (door-to-door, wild life immunization model -WIM-, parenteral vaccination, and combinations of different vaccine delivery systems) should be included 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 a operational basis (i.e. owned dogs, dogs accessible by parenteral vaccination, free-roaming dogs etc). To compare the results of a study with another or allocating a dog to a given category criteria used need to be precisely described. In order to study certain aspects of bait delivery, methods recommended by WHO should be revised for better estimating dog population parameters.

In due course, WHO should prepare detailed recommendations for the use of oral vaccination in dogs using the WHO recommendations provided in the 8th report of the Expert Committee on Rabies (Technical Report Serie n°824, WHO 1992, section 10.4.3) as a guide.

It is important that WHO maintains inventories of vaccine delivery studies accompanied by detailed information on the circumstances in which those studies have been carried out. In addition, it is recommended that the results of such studies be published in appropriately accessible, refereed journals.

WHO should prepare a review of published and unpublished relevant studies to summarize the achievements in the field of oral vaccination of dogs against rabies.

5.3 VACCINE BAIT DELIVERY

The group defined efficacy as protection of a vaccinated animal after challenge. Immunogenicity as measured by antibody response was considered as only a part of the measurement of efficacy.

The group felt it was important to examine the following:

Vaccine efficacy when administered by instillation; efficacy of vaccines when given in a bait (vaccine-in-bait efficacy); efficacy of vaccine baits in the field; recommendations of the previous consultation reports were examined, as well as the achievements of subsequent studies aiming at fulfilling these recommendations for each vaccine type; and the constraints limiting these achievements.
The recommendations on the administration of vaccine to captive animals by oral instillation were considered adequate. However, recommendations on how to carry out vaccine efficacy studies when incorporated in a bait were missing. Therefore the following recommendations were made:

! Vaccine-bait efficacy studies should be preceded by studies on the efficacy of the vaccine given by direct instillation and bait acceptability tests as described in previous recommendations.

! 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 should not necessarily be standardized.

! The vaccine-bait combination should have been previously tested in an indigenous dog population. When indigenous dogs have previously been tested in a different context, the validity of these results in the local situation should be carefully assessed.

! The administration of vaccine-baits should conform with bait acceptance trials as previously recommended.

! 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 (e.g. 8 hours) and, if possible, the time of consumption should be recorded.

! In pilot evaluations of oral vaccination efficacy it is important that effective surveillance procedures be in place to assess the effect and the outcome of the programme. This is also desirable in programmes based on parenteral vaccination.

When examining whether efficacy studies had followed the recommended WHO Guidelines, it was seen that only certain studies with VRG had fulfilled them. Studies on SAG$_2$ had almost fulfilled these, although, at the time this consultation was held no studies had been done on local dog types, which is one of the prerequisites for a complete vaccine efficacy study. No study with any other vaccine had fulfilled all the requirements.

Regarding the efficacy of an oral vaccination campaign, the group felt that no recommendation had been made on the conduction of pre- and post-campaign surveys. These are necessary to determine the effect of the campaign.

**ANNEX 1**

**LIST OF PARTICIPANTS**
ANNEX 2

PRIOR RECOMMENDATIONS ON EFFICIENCY, DOG BAIT PREFERENCE TEST AND SAFETY

Excerpt from the 2nd WHO Consultation on Oral Immunization of Dogs against Rabies, 6 July 1990 (WHO/Rab.Res./91.37).

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

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

Excerpt from the 4th WHO Consultation on Oral Immunization of Dogs against Rabies, 14-15 June 1993 (WHO/Rab.Res./93.42)

6.1.1 Standardized test for determining dog bait preferences

A standardized test method for determining dog bait preferences and efficacious vaccine delivery to the oral cavity of dogs should be developed and used, where possible, to provide a common basis of comparison between different field studies and investigators. The 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 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 any bait candidate in a field trial first by the means of the tracking-station method, before further use either for the evaluation of bait delivery systems or for oral mass vaccination in the field on a large scale.

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

This type of bait should be clean, easy to handle, and should fulfil the requirements made for foodstuffs so that dog owners would not find them objectionable to handle. It may be impracticable to distribute chicken baits to dog owners. Existing artificial baits, however, 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 baits or the Köfte bait may be useful. The use of artificial baits for this purpose should not be excluded. However, their design should respect the dog’s food intake behaviour and food preferences.

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

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

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

6.3.1 For modified live vaccines:

(a) laboratory tests:

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

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

- In addition, where feasible, at least 10 and if possible 50 of each of the most common local rodent species should be given the field dose of vaccine (i.e. the dose which is contained in a bait) orally and intramuscularly (this may require 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.

- Relevant local wild or domestic animal species that may take baits should also be given a dose of vaccine 10 times orally the field concentration in a volume adapted to body weight.

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

(b) monitoring human exposure and providing care

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

6.3.2 Recombinant live virus vaccines (RLV)

(a) laboratory tests

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

- Innocuity can be expected from vaccine strains where either genomic deletions or insertional mutagenesis has led to the inactivation of virulence-relevant gene(s). It has been shown, for instance, that inactivation of the thymidine kinase gene leads to vaccinia virus mutants of reduced virulence; similar approaches should be followed for other candidate vector viruses.
Here, other safety aspects are considered, i.e. the safety of a vaccine for non-target species and for humans. Whenever possible, the risks of vaccine virus transmission to humans (e.g. via an immunosuppressed non-target species or a dog re-excreting the vaccine strain) should be evaluated.

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

(b) monitoring human exposure

In the case of vaccinia vectored rabies glycoprotein vaccine (as now developed) where the rabies glycoprotein gene is inserted at the thymidine kinase position in the vaccinia DNA, this vaccine may be considered non-infectious for rabies, and pre-exposure or postexposure rabies vaccination is not recommended for persons exposed to this vaccine. However, persons exposed to the vaccinia recombinant rabies virus should be followed-up. Paired sera should be obtained following the exposure and afterwards (e.g. 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 bases as such vaccines are developed.

A hypothetical risk is the recombination of the RLV vaccine viral genome with that of another virus, with the resulting recombinant 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’s to recombine, to cause persistent infections, or to become oncogenic should be kept in mind and investigated. This applies especially to other potential vector viruses whose DNA replication is in the nucleus.

6.3.3 General considerations on risk

In summary, a safe vaccine virus candidate should:

- not acquire virulence during replication in the vaccinee;

- not be oncogenic in the vaccinee;

- should fulfil the requirements for target and non-target species established in this document (see Sections 6.3.1 and 6.3.2);
- not recombine with viruses occurring in nature to result in viable pathogenic progeny;

- demonstrate that its possible excretion is not hazardous;

- be evaluated for potential public health risks associated with its use;

- bear at least one genetic marker for identification.

6.3.4 Special safety considerations

- the use of live vaccines should be discouraged when the risk of unintentional exposure to severely immunocompromised populations is deemed high, because of the chance of enhanced viral replication, altered tropism or untoward adverse events; conversely, inactivated vaccines do not appear to represent a danger to immunocompromised individuals.

   Research on inactivated oral rabies immunogen for dogs is in progress. Some encouraging results have been obtained in raccoons. However, future results will require careful assessment before the concept of an inactivated rabies oral immunogen for dogs is abandoned as the next obvious generation of vaccine. In lieu of an alternative, several candidate attenuated or recombinant rabies viruses appear to offer safe, efficacious and economical solutions for the oral immunization of immunocompetent hosts.

   - The candidate live vaccine should also be tested in primates, such as chimpanzees, baboons, rhesus monkeys, etc. At least 10 animals of one species should be given 1 ml or more of 10 times the intended field dose of vaccine by direct instillation into the oral cavity. Whenever sufficient numbers of animals are available, the vaccine should also be tested in a similar number of immunocompromised primates. No vaccine related mortality should occur during an observation period of at least 90 days. Tests for rabies and vector virus antibody should be made before inoculation of the vaccine and at the end of the experiment.

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

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

   - Levels of vaccine virus excretion during the days after oral vaccination should be determined. An appropriate quantitative test should be established for that purpose. Subsequent experiments should involve appropriate immuno-suppressed models to determine the effects of these virus titres.
Excerpt from the 5th WHO Consultation on Oral Immunization of Dogs against Rabies, 20.22 June 1994 (WHO/Rab.Res./ 94.45)

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 : 6.3.1, a), namely:

- 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 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 with the examination of relevant major organ systems 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 Special safety considerations (Swab Test Recommendations)

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” (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 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.
Table 1: Safety of oral vaccines for dogs

Tests performed(+) or not (-) on candidate vaccines (under laboratory conditions)

<table>
<thead>
<tr>
<th>Vaccine candidates</th>
<th>in wild rodents (most common species)</th>
<th>in primates (immuno-competent)</th>
<th>in non-target species (other than rodents)</th>
<th>in pupples</th>
<th>in immunosuppressed animal models</th>
<th>overall rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCID</td>
<td></td>
</tr>
<tr>
<td>1. RLV - VRG</td>
<td>+ (European and North American species)</td>
<td>+ (squirrel monkeys ID) (Chimp. oral)</td>
<td>+ (in over 50 mammalian &amp; avian species)</td>
<td>NT</td>
<td>+ (orally) (+/- parenterally)</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nude mice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other models</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FIV &amp; FeVL Cats</td>
<td></td>
</tr>
<tr>
<td>2. MLV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- SAG1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(European species)</td>
<td></td>
<td></td>
<td></td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>- SAG2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(European &amp; African species) (field trials)</td>
<td></td>
<td></td>
<td></td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>- SAD B19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ (orally) (95% death other routes)</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td>(Chimp. orally)</td>
<td></td>
<td></td>
<td></td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>- SAD PS/88</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
<td>+/- (orally) (83% deaths I.M.)</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td>(baboons orally)</td>
<td></td>
<td></td>
<td></td>
<td>+ (orally) (100% deaths I.M.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Some</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(66% death I.M.)</td>
<td></td>
</tr>
</tbody>
</table>

RLV = Recombinant live vaccine
MLV = Modified live vaccine
Table 2: Immunogenicity and efficacy of oral vaccines for dogs

Tests performed (+) or not (-) on candidate vaccines

<table>
<thead>
<tr>
<th>Vaccine candidates</th>
<th>Immunogenicity</th>
<th>Efficacy</th>
<th>Overall rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Instillation</td>
<td>via one bait</td>
<td>Instillation</td>
</tr>
<tr>
<td>1. RLV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- VR-G</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- HAV5RG</td>
<td>+ (Ad5. δ E3-RG1)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>- RPRG</td>
<td>- (33 to 50%)</td>
<td>NT</td>
<td>+ (364 days)</td>
</tr>
<tr>
<td>2. MLV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- SAG1</td>
<td>+</td>
<td>+</td>
<td>+ (364 days)</td>
</tr>
<tr>
<td>- SAG2</td>
<td>+</td>
<td>+</td>
<td>+ (180 days)</td>
</tr>
<tr>
<td>- SAD B19</td>
<td>+ (78-100%)</td>
<td>+ (61% in field dogs)</td>
<td>- (60% mortality in controls)</td>
</tr>
<tr>
<td>- SAD Berne</td>
<td>+ (100%)</td>
<td>NT</td>
<td>+ 4 months</td>
</tr>
<tr>
<td>- SAD P5/88</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>- ERA</td>
<td>+ (100%)</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

RLV = Recombinant live vaccine  
MLV = Modified live vaccine  
NT = Not tested
<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Year of report</th>
<th>Mode of vaccine application, types of dog and vaccine titres &amp; volumes</th>
<th>Number of animals tested</th>
<th>Number of controls</th>
<th>Titre of challenge</th>
<th>Results of challenge test</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAG1</td>
<td>1992</td>
<td>1. vaccine instillation</td>
<td>6</td>
<td>6</td>
<td>$10^{5.8}$MICLD$_{50}$</td>
<td>0/6 at day 364</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1 in laboratory dogs</td>
<td></td>
<td></td>
<td></td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6$TCID$_{50}$/1 ml</td>
<td></td>
<td></td>
<td></td>
<td>6/6 at day 364</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^7$TCID$_{50}$/1 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^8$TCID$_{50}$/1 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAG2</td>
<td>1993</td>
<td>$10^6$PFU/1 ml</td>
<td>5</td>
<td>5</td>
<td>$10^{5.6}$MICLD$_{50}$</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6$PFU/1 ml</td>
<td></td>
<td></td>
<td></td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6$PFU/1 ml</td>
<td></td>
<td></td>
<td></td>
<td>4/5 at day 100 pV</td>
</tr>
<tr>
<td>SAG2</td>
<td>1994</td>
<td>$10^9$TCID$_{50}$/1 ml</td>
<td>10</td>
<td>10</td>
<td>$10^{5.8}$MICLD$_{50}$</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^9$TCID$_{50}$/1 ml</td>
<td></td>
<td></td>
<td></td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. via one bait</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>2.1 in laboratory dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6$TCID$_{50}$/1 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAG1</td>
<td>1990</td>
<td>$10^6$PFU (10$^5$MICLD$_{50}$)</td>
<td>6</td>
<td>6</td>
<td>$10^{5.8}$MICDL$_{50}$</td>
<td>1/6</td>
</tr>
<tr>
<td>SAG2</td>
<td>1994</td>
<td>$10^7$TCID$_{50}$/per bait</td>
<td>10</td>
<td>10</td>
<td>$10^{5.8}$MICDL$_{50}$</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^8$TCID$_{50}$/per bait</td>
<td></td>
<td></td>
<td></td>
<td>0/10</td>
</tr>
</tbody>
</table>

$pV$ = post-vaccination
Table 4: VR-G efficacy studies

<table>
<thead>
<tr>
<th>Year of report</th>
<th>Titre</th>
<th>Number of animals tested</th>
<th>Number of controls</th>
<th>Titre of challenge</th>
<th>Results of challenge test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>1. Vaccine instillation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1 In laboratory dogs</td>
<td>$10^4$PFU/1 ml</td>
<td>4</td>
<td>5</td>
<td>$10^3$MICLD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>$10^4$PFU/1 ml</td>
<td>4</td>
<td>5</td>
<td></td>
<td>4/4</td>
</tr>
<tr>
<td>1993</td>
<td>$10^3$TCID&lt;sub&gt;50&lt;/sub&gt;/1 ml</td>
<td>4</td>
<td>5</td>
<td>$10^6$MICLD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0/4 (69 days)</td>
</tr>
<tr>
<td></td>
<td>$10^3$TCID&lt;sub&gt;50&lt;/sub&gt;/1 ml</td>
<td>4</td>
<td>5</td>
<td></td>
<td>2/4 (69 days)</td>
</tr>
<tr>
<td>1993</td>
<td>$10^3$TCID&lt;sub&gt;50&lt;/sub&gt;/1 ml</td>
<td>6</td>
<td>5</td>
<td>$10^6$MICLD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0/6 (4 mths.)</td>
</tr>
<tr>
<td></td>
<td>$10^3$TCID&lt;sub&gt;50&lt;/sub&gt;/1 ml</td>
<td>6</td>
<td>5</td>
<td></td>
<td>1/6 (4 mths.)</td>
</tr>
<tr>
<td>1993</td>
<td>1.2 In caged local (Tunisian) dogs</td>
<td>6</td>
<td>5</td>
<td>-</td>
<td>0/6 (4 mths.)</td>
</tr>
<tr>
<td></td>
<td>$10^3$TCID&lt;sub&gt;50&lt;/sub&gt;/per bait</td>
<td>6</td>
<td>5</td>
<td></td>
<td>1/6 (4 mths.)</td>
</tr>
<tr>
<td>1993</td>
<td>2. via a bait in laboratory dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 One bait only</td>
<td>$10^3$TCID&lt;sub&gt;50&lt;/sub&gt;/per bait</td>
<td>6</td>
<td>8</td>
<td>$10^6$MICLD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0/3 (7 mths.)</td>
</tr>
<tr>
<td></td>
<td>$10^3$TCID&lt;sub&gt;50&lt;/sub&gt;/per bait</td>
<td>8</td>
<td>9</td>
<td>idem</td>
<td>0/3 (13 mths.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/3 (10 mths.)</td>
</tr>
<tr>
<td></td>
<td>$10^3$TCID&lt;sub&gt;50&lt;/sub&gt;/per bait</td>
<td>5</td>
<td>9</td>
<td>idem</td>
<td>0/3 (6 mths.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0/2 (13 mths.)</td>
</tr>
<tr>
<td>2.2 Two baits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4/4 (6 mths.)</td>
</tr>
<tr>
<td>2.3 Three baits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5/5 (13 mths.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4/4 (6 mths.)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0/1 (13 mths.)</td>
</tr>
</tbody>
</table>

(*) = time of challenge expressed in days or months after vaccine administration

* 1 died before challenge
Table 5: Immunogenicity and efficacy studies of other vaccines

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Year of report</th>
<th>Vaccine titre</th>
<th>Seroconversion rate</th>
<th>Challenge test</th>
<th>number of deaths/animals vaccinated</th>
<th>number of deaths/controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>by instillation</td>
<td>via bait</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPRG 1992</td>
<td>1992</td>
<td>$10^6$PFU</td>
<td>50% (3/6)</td>
<td>NT</td>
<td>0/6</td>
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<tr>
<td></td>
<td></td>
<td>$10^5$PFI</td>
<td>33% (2/6)</td>
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<td>2/6</td>
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<tr>
<td>SAD B19 1988</td>
<td></td>
<td>$10^4$MICDL$_{50}$</td>
<td>0% (0/5)</td>
<td>NT</td>
<td>6/7 (lab. dogs)</td>
<td>0/14</td>
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<tr>
<td></td>
<td></td>
<td>$10^3$MICDL$_{50}$</td>
<td>80% (4/5)</td>
<td></td>
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<tr>
<td></td>
<td>1992</td>
<td>?</td>
<td>100% (5/5)</td>
<td></td>
<td>1993</td>
<td></td>
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<tr>
<td></td>
<td>2 x $10^5$FFU</td>
<td>78% (11/14)</td>
<td>(street dogs)</td>
<td></td>
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<tr>
<td></td>
<td>1992</td>
<td>$10^6$FFU/ml</td>
<td>100% (17/17)</td>
<td></td>
<td>1994</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 x $10^7$FFU/ml</td>
<td></td>
<td>61% (25/41) (street dogs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAD Berne</td>
<td>1992</td>
<td>$10^7$TCID$_{50}$/ml</td>
<td>100% (7/7)</td>
<td></td>
<td></td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^7$TCID$_{50}$/ml</td>
<td>100% (7/7)</td>
<td></td>
<td></td>
<td>4/4</td>
</tr>
<tr>
<td>ERA 1988</td>
<td></td>
<td>?</td>
<td>100% (10/10)</td>
<td></td>
<td></td>
<td>0/7</td>
</tr>
<tr>
<td>1990</td>
<td>$10^6$MICDL$_{50}$/ml</td>
<td></td>
<td>via a CH</td>
<td></td>
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