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Field Application of Oral Rabies Vaccines for Dogs:

**Report of a WHO Consultation
organized in collaboration with the
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**Geneva, Switzerland
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WORLD HEALTH ORGANIZATION

**Zoonotic Diseases
Division of Emerging and other Communicable
Diseases Surveillance and Control**

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1. INTRODUCTION

With field application of the oral vaccination technique and production of commercial dog specific vaccine baits in sight, this consultation is seen as the first with the objective to elaborate the framework for OVD in the field. More precisely, the missions identified were to:

- review the rabies situation and need for oral immunization of dogs in invited countries;
- review advances in vaccine and bait development;
- review, amend and possibly endorse a document entitled: “Guidelines for research on oral rabies vaccines and field application of oral vaccination of dogs against rabies”;
- establish criteria for identification of country and trial site;

Dr. F.-X. Meslin welcomed the participants. He thanked scientists and commercial partners for their continued efforts in the characterization and development of OVD tools, such as vaccine strains and vaccine baits, as well as bait delivery techniques and expressed his wish for continued productive discussions in the future.

Dr. A. I. Wandeler was elected Chairman and Dr. C. L. Schumacher was nominated Rapporteur.

2. RABIES SITUATION AND EXPECTED ROLE OF THE OVD IN SELECTED DOG RABIES INFECTED COUNTRIES

2.1 Indonesia

Until 1989, rabies was endemic in Indonesia with approximately 250 human rabies cases and 1,500 animal cases diagnosed each year. Dogs were responsible for 98% of the human cases and for 22,000 to 24,000 recorded animal bites. Sixteen thousand post-exposure treatments were administered annually. Rabies control efforts included dog elimination and mass vaccination of dogs but no clear success was visible until the efforts of the three national departments involved in rabies control were coordinated in 1989. Since then, approximately 2 million rabies vaccine doses, of which three quarters are locally produced, have been administered to dogs. Vaccine administration to dogs is organized in a decentralized manner, with one teacher in every village responsible for dog census, handling and vaccine application. Vaccination campaigns were generally started in areas at higher risk of allowing spread of the disease to adjacent regions. Between 1990 and 1997, the number of reported human rabies cases in endemic areas decreased from 62 to 43 and laboratory confirmed cases in animals from 1,570 to 859. Animal bites, recorded at over 17,000 in 1990, dropped below 15,000 in 1997 and a reduction in post-exposure treatments from around 11,000 to less than 7,000 was observed over the same period of time. This downward trend is expected to continue in 1998. However, whereas on certain islands of the Indonesian archipelago, such as Java, rabies elimination may be achievable soon, the problem persists on others. It is hypothesized that in these areas, notably West Sumatra and Kalimantan, accessibility of dogs to parenteral vaccination is low due to cultural or religious beliefs and misconceptions about side effects of

parenteral vaccination on dogs. It is further believed that oral rabies vaccines for dogs may provide a viable solution to this problem. Based on an assumed dog to human ratio of 1:16, the dog population of Indonesia should be around 12 million. The authorities estimate that approximately 615,000 vaccine baits would initially be required in the problem areas.

2.2 Mexico

Rabies is endemic in Mexico and the dog continues to be the main reservoir of rabies. However, the number of cases has declined since 1990, with a direct correlation observed between laboratory confirmed rabies cases in dogs (3,049 in 1990 and 521 in 1997) and humans (72 in 1990 and 23 in 1995). Based on an assumed dog to human ratio of 1:6 or 1:7, the Mexican dog population is presently estimated to be 13-16 million. Of the 23 rabies human cases reported in 1995, 20 were caused by dogs, 2 by vampire bats (*Desmodus rotundus*) and one by a fox. In 1998, eighty to ninety thousand people were bitten by dogs and 26,000 people received post-exposure treatment. Because puppies are responsible for about 65% of human cases, puppies are vaccinated starting at 1 month of age. The decline of animal and human rabies cases since 1990 has mainly been attributed to parenteral dog mass vaccination campaigns. Over 12.3 million doses of parenteral canine rabies vaccine will have been administered in 1998 during 1-2 national vaccination weeks with an additional 900,000 doses applied by private veterinarians. The goal is to reduce the number of human cases to 15 at the end of 1998 and to 10 by the year 2000. Ideally, none of these cases should be caused by dogs (urban rabies). Around 20% of Mexico's dog population is considered stray or free roaming. Accessibility to parenteral vaccination is presumed low in this group and therefore oral vaccination is seen as a viable alternative. OVD could be especially useful in selected areas and locations where large numbers of free-roaming dogs congregate, such as public markets, garbage dumps, and periurban areas, or where no centres for canine rabies control exist and in border areas.

2.3 Thailand

Rabies in humans and animals is endemic in all regions of Thailand although with varying degrees of incidence. Human rabies deaths were mainly reported (84% of the total in 1997) from the central and northeastern parts of the country. Dogs account for 95% of positive rabies diagnostic results while the remaining comprise cats, monkeys, cattle, pigs, horses, gibbons and very infrequently, rodents and small wild animals. Positive samples in animals have decreased from 6,535 in 1990 to 3,783 in 1994 and 1002 in 1997. Human rabies cases in Thailand have declined from 370 in 1980 to 56 in 1997. This reduction in human rabies deaths has been correlated with the increasing availability of tissue culture vaccines used for post-exposure treatments (PET) (160,448 in 1994). An epidemiological PET survey in 1993 revealed that children (<12 years old) represented 51% of the total treatments. Most of the patients (74 %) received PET within 48 hours of exposure. Vaccines were mostly, (70.4%), given intramuscularly according to the 5 dose «Essen» regimen. Full post-exposure treatment including ERIG is received by about 10% of the patients treated. Other important activities related to human rabies control are delivery and cold chain monitoring of rabies vaccines, training courses for physicians and health education teachers, training of one health care volunteer per village in dog bite treatment, dog vaccination and dog population surveys, as well as public education campaigns including mass media broadcasting. Community participation is considered to be a key to success.

The dog is the main transmitter of rabies to humans (96%). Dog rabies control measures in Thailand include annual mass parenteral vaccination campaigns and dog population control by hormone injections (500,000-700,000 female dogs per year) and neutering (180,000 annually, predominantly male dogs). Since 1993, approximately 4.9 million dogs have been vaccinated against rabies each year. However, vaccination coverage is unlikely to exceed 60% in a dog population estimated at 7 and 6 million animals in 1993 and 1997, respectively. In spite of these efforts and the gradual decrease of animal and human cases, rabies has remained endemic almost throughout Thailand. Official sources provide two main explanations for this: a large stray dog population (0.5 to 1 million dogs) which is inaccessible to rabies vaccination or elimination campaigns (the latter is unpopular in a predominantly Buddhist country), and the obligation to pay a vaccination fee. To reach the target of 80 % vaccination coverage and « no human rabies death by the year 2000 », the Thai authorities contemplate using oral rabies vaccination and population control through hormone injection via dart gun to the stray dog population and to seek funding from non-governmental organizations to cancel vaccination fees.

2.4 Tunisia

Canine rabies remains a serious public health problem in Tunisia. The recent history of this disease can be divided into four periods characterized by variations in incidence of the disease. During the first period, from 1960 to 1981, the annual average number of cases reached 207 in animals and 18 in humans. These figures fell to 91 and 5 respectively during the second period, from 1982 to 1987, with zero human cases in 1985. During the third period, from 1988 to 1992, the annual average number of cases was 281 for animals and 10 for humans with zero and 25 human cases in 1988 and 1992, respectively. During the fourth period, from 1993 to 1997, an average number of cases of 148 in animals and 5 in humans were recorded. Contrary to the first period which was characterized by the absence of a national rabies control programme based on vaccination of dogs, control improved during the second period because of the introduction of a national programme, with the technical assistance of WHO, which resulted in the favourable figures obtained during this period. This programme was based on epidemiological surveillance, mass campaigns for parenteral immunization of dogs, awareness campaigns, post-exposure treatment of humans and elimination of free roaming dogs at night. During the third period, the simultaneous occurrence of several events in animal health in Tunisia, such as the major epizootic of foot-and-mouth disease of 1989-1990 and the decentralization of veterinary services, led to a temporary slow-down of mass vaccination campaigns and consequently, a recrudescence of rabies in the country. In 1992, 581 animal cases and 25 human cases were recorded. This recrudescence was also caused by the lack of precise ecological data on the dog population before the beginning of the national programme, its short duration, the absence of a serious follow-up, due in part to a limited community participation and a lack of coordination between the different sectors. The fourth period was characterized by an increased effort to control the disease. However, despite the political willingness expressed through the creation of national and regional interdepartmental commissions for the coordination of the national rabies control programme, and the motivation of the field staff in charge, the number of animal and human cases has remained high.

Studies conducted on dog population dynamics, the accessibility of dogs to parenteral immunization and the mode of supervision of dogs by their owners, have shown that 15-25% of Tunisian owned dogs are not easily accessible to parenteral vaccination. As a recent serological study in Tunisia showed that only 88% of vaccinated dogs yielded virus neutralizing antibody titers ≥ 0.5 IU/ml and since evidence has accumulated that annual mass vaccination campaigns do not reach more than 70% of the total dog population, only 62% of the total owned dog population exhibit an antibody titer in response to the single annual vaccination. Taking into account dogs with undefined ownership status, the actual vaccine coverage in the total dog population is suspected to be significantly lower. High population turnover rates and heterogeneous vaccination coverage are responsible for a significant proportion of unvaccinated dogs that are especially at risk, to contract and transmit rabies. To overcome the insufficiencies of parenteral immunization, dog accessibility to oral vaccination, safety and efficacy of oral vaccine candidates, bait acceptance and delivery systems have been studied with promising results in collaboration with WHO since 1988. Although parenteral vaccination remains a determinant for the success of a national dog rabies control programme, other elements should be included such as enhanced community participation, international cooperation, allocation of sufficient resources and dog vaccination using safe and efficient oral vaccines as a complementary means of immunization.

2.5 Turkey

Dog rabies prevails in Turkey, but several rabies cases have also been confirmed in wildlife. Between 1988 and 1997, 2743 (76.2%) of all registered rabies cases were in dogs and only 43 (1.2%) cases were diagnosed in wildlife. Nowadays, rabies is becoming an increasing problem in urban areas due to the growing number of urban dog populations as a result of the migration of the rural human population to the major cities since the late 1970s.

In the last decade, the rabies incidence decreased significantly in the whole country. Between 1988 and 1997, the number of animal rabies cases dropped from 710 to 142. This decrease was observed not only in dogs but in all domestic animals. In 1988, 546 rabid dogs were registered, whereas the number was 117 in 1997. Rabies cases in cattle also decreased within this period. Currently, the highest rabies incidence is observed in the Province of Istanbul where, in contrast to other areas, the number of rabies cases increased between 1994 and 1996.

In the late 1980s, the national authorities initiated several rabies control programmes. Methods used included the control of stray dog populations, dog vaccination, quarantine, public information and training. Removal and destruction of stray dogs has been met with increasing public opposition and hence shelters and neutering programmes have been set up. Local production of dog rabies vaccine doses (Kelev strain) decreased from 1987 to 1997 (400 000 to 170 000 respectively). Imported rabies vaccines are also available. The decrease in vaccine doses reflects the reduction in rabies incidence countrywide since the initiation of the control programme. To limit the geographical spread of the disease, movement of animals (pets and livestock) is restricted for 6 months from areas where a rabies case has been diagnosed. Public education about the disease through mass media and in schools is an important element of the programme.

Elimination of rabies in Turkey appears possible in the near future. However, due to the increasing dog population, future rabies control activities should focus on the promotion of responsible dog ownership and intensified parenteral vaccination campaigns in areas where rabies is still present. In 1992, a project to test the feasibility of oral vaccination of dogs against rabies was started in Turkey. The method may be included into future control strategies.

2.6 South Africa

Canine rabies in South Africa is at present largely confined to three provinces on the eastern coast of South Africa. In the rest of the country, the disease is mainly spread by wild animal vectors such as the yellow mongoose, the black-backed jackal and the bat-eared fox. Dog vaccination remains a very high priority in the prevention of rabies in humans since nearly all human rabies fatalities over the past 20 years have been attributable to dogs. Dog immunization is usually carried out at central point vaccination clinics (300,000 to 400,000 vaccine doses administered per year) following intense publicity and public awareness campaigns.

Coordination of efforts has improved considerably following the amalgamation of the many veterinary departments (and their areas of jurisdiction) which existed prior to 1994. The number of cases in dogs has decreased from 410 in 1995 to 250 in 1996 and 226 in 1997.

This and the improved cooperation of the veterinary and medical professions have both contributed to the decrease in human deaths from an all-time high of 29 in 1995 to only 6 in 1997. Pre- and post-exposure treatments are based on WHO guidelines. Developmental work has commenced on the oral vaccination of dogs using SAG2 vaccine and it is hoped to use this approach to supplement parenteral vaccination in the future.

2.7 Sri Lanka

Rabies is endemic in Sri Lanka. Over 96% of animal rabies cases are diagnosed in dogs whose population is estimated to be approximately 2.3 million based on an assumed dog to human ratio of 1:8. Fifteen to twenty percent of this population are considered stray or community dogs. Dogs are responsible for 97% of human rabies cases, 64 % of which are attributed to stray dogs. Control strategies adopted in Sri Lanka include parenteral immunization of dogs, elimination of stray dogs, post-exposure prophylaxis for bite victims, public health education, promotion of community participation and multi-sectoral cooperation. Other components such as legislation and monitoring of rabies are also important. Since the initiation of the dog vaccination programme in the early seventies, the number of vaccine doses administered have steadily increased and reached 400,000 to 600,000 annual doses after 1990. Due to control measures, the numbers of human rabies cases has decreased from 377 in 1973 to 98 cases in 1993. However an increase is currently reported as 135 cases were notified in 1997. The major problem is that the immunization coverage achieved lies significantly below the target of 75%. It is estimated that only 70-75% of the owned dog population are presented for vaccination and the stray population is not reached at all. Dog vaccination coverage is thus heterogeneous ranging from an estimated 55-75% depending on the location. In addition, mass vaccination campaigns cannot be repeated frequently enough to counter the decrease in herd immunity due to the dog

population turnover. In certain areas, where a high incidence of rabies was continuously reported, removal of unvaccinated dogs immediately after vaccination campaigns was adopted in 1995. The use of goat brain vaccine for human post-exposure treatment was finally abandoned recently and an intradermal PET regimen with cell-culture vaccine was introduced. As a result of all these activities, 29 human rabies cases have been reported during the first half of 1998, compared to 72 cases during the same period in 1997. Since 1996, oral vaccination has been investigated as an additional means of rabies control. Initial results on the acceptance of placebo baits have been promising (see 3.2.3).

3. REVIEW OF:

3.1 Vaccine Safety and Efficacy

3.1.1 Modified live virus vaccines

-SAG2

Safety of the vaccine strain SAG2 and efficacy of the vaccine bait DBL2 in mongrel dogs

Safety of the SAG2 rabies virus vaccine in dogs and several non-target species and its efficacy in dogs were evaluated in Tunisia. For testing safety, 21 dogs (8 adult, 3 juveniles and 10 puppies) and 15 cats (obtained from animal owners), as well as 8 jackals (captured in the wild) each received, orally, one ml of liquid vaccine titrating $10^{9.4}$ TCID₅₀ per ml. In addition, 62 wild rodents received intramuscularly or orally according to their size, 0.1 ml or 0.3 ml of SAG2 vaccine suspension. No animal had detectable levels of virus neutralizing antibody (VNA) titres prior to vaccination as determined by the Fluorescent Antibody Virus Neutralization test (FAVN) [OIE Manual of Standards for Diagnostic Tests and Vaccines, OIE, 1996, pp. 211-213]. On day 90, 16 of 21 surviving dogs were euthanized, while 5 were kept for further observation. Of these, one juvenile dog and one puppy still had VNA titres exceeding 0.5IU/ml on day 150 and 180, while three other puppies were negative. Only one cat had positive titres detected at day 30 and 90. Sera collected at day 30 and 90 from the three jackals had positive titres, although they were low in two animals. Twenty-three of the twenty-nine sera collected from wild rodents had positive titres 60 days post vaccination. No animal (dogs, cats, jackals or rodents) showed clinical signs of rabies and rabies virus antigen was not detected in any of the samples taken from the brain or the salivary glands of dead or euthanized animals when examined by the FAT. The presence of vaccinal rabies virus was detected in the saliva of three cats, 6 hours, but not 3 days after oral vaccine instillation.

For efficacy testing, seven Tunisian mongrel dogs were each given one dog bait (DBL2) containing $10^{8.3}$ TCID₅₀ of the attenuated rabies vaccine strain SAG2. All dogs consumed the bait at least partially. During the 30 days following vaccination only two of seven vaccinated dogs exhibited VNA titres ≥ 0.5 IU/ml. Nevertheless, five of seven vaccinated animals survived a challenge with a Tunisian canine street rabies virus which killed five of the six controls.

Efficacy of a SAG2 rabies vaccine administered orally via baits in laboratory dogs

A dose response experiment was conducted in laboratory dogs in the USA to compare the efficacy of a single liquid or lyophilized dose of commercial SAG2 rabies virus vaccine at dilutions containing between $10^{8.3}$ and $10^{6.9}$ TCID₅₀.

Three groups were included:

- 13 dogs (3-5 per dilution) that received liquid SAG2 vaccine (either $10^{7.0, 7.5}$ or 8.2 TCID₅₀) via a bait
- 13 dogs (4-5 per dilution) that received lyophilized SAG2 vaccine ($10^{6.9, 7.2, 8.3}$ TCID₅₀) via a bait (DBL-2)
- 5 dogs received no vaccine and served as controls.

All adult dogs were obtained from a commercial source and were seronegative (<1/5) for rabies VNA prior to inclusion in the study, by the Rapid Fluorescent Focus Inhibition Test (RFFIT) [Laboratory Techniques in Rabies, Fourth Edition, WHO, 1996, Chapter 15, pp. 184]. They were offered a single bait and were observed for 4-6 weeks until challenge into the masseter muscle with 0.5 ml ($10^{5.3}$ MICLD₅₀) of a street rabies virus of coyote origin.

No adverse events due to vaccine were observed in more than 3,160 dog days of observation. VNA were first detected 14 days post-ingestion. Seroconversion was observed by day 14 in 7 dogs vaccinated with liquid SAG2 (4 of 5 in the highest concentration, 1 of 3 in the next dilution, and 2 of 5 in the lowest dilution) and 5 dogs vaccinated with lyophilized SAG2 (3 of 5 in the highest concentration, 2 of 4 per next dilution, but none in the lowest dilution). There was no apparent difference in VNA GMT between vaccine groups and none in efficacy between vaccines, with >70% protection overall. All 5 controls succumbed to challenge, as did 4 of 13 dogs in the liquid SAG2 group (2 of 5 in the lowest dilution, and 1 each per the other two dilutions) and 3 of 13 from the group vaccinated with lyophilized SAG2 (all from the lowest dilution of vaccine). All dogs that had detectable VNA at any time survived, regardless of their titre on the day of challenge, including at least 4 previously seropositive dogs that became seronegative by the day of challenge. Three vaccinated dogs survived without any detectable VNA (all at <7.5 dilution). All survivors had detectable VNA by day 7 post-challenge.

Safety and excretion of SAG2 in puppies

SAG2 containing 10^9 TCID₅₀ was administered by the oral or intramuscular route to 2 groups of 10 indigenous South African puppies, each aged 7 to 10 weeks. None of the animals developed clinical signs which could be related to vaccine administration during the 120 days observation period. SAG2 virus was isolated from 7 of 10 saliva swabs taken 1 hour after oral vaccine administration and from 8 of 10 saliva swabs taken 1 hour after intramuscular inoculation. In a separate trial of virus, evidence was accumulated that the presence in the saliva of parenterally vaccinated puppies was due to contamination. No virus was isolated from saliva samples from either group taken at 7 hrs, 24 hrs and daily for 7 days thereafter.

VNA were detected by the FAVN test in 17 of 20 puppies within 7 days of vaccination. Ten puppies dosed orally with SAG2 yielded the highest antibody titer by day 60, in contrast to those injected, which peaked at 30 days. Whereas all parenterally injected puppies still had significant antibody titers on day 120, 3 of the 10 orally inoculated animals had fallen to pre-trial levels. No virus was isolated or antigen detected in brain, tonsils or salivary gland samples collected from the euthanized puppies at the end of the trial (day 120).

-SAD B19

Dissemination of SAD B19 in foxes and dogs

The presence of SAD B19 was investigated in a number of tissues and secretions (cerebellum, cerebral cortex, digastric muscle, oesophagus, hippocampus, lungs, mandibular lymphnodes, mucosa of small intestines, nasal mucosa, salivary glands and nasal secretions as well as saliva) from a total of 6 dogs. Two dogs were sampled on day one, 2 on day four and 2 on day 8 after oral inoculation of $10^{8.3}$ FFU. The vaccine virus was not detected in saliva or other samples with the exception of a dog small intestine from which virus was recovered 1 day post vaccine instillation. The sensitivity of the reisolation technique on BSR cells was 1 FFU per 50-100 mg of tissue and 0.5 ml for saliva respectively. Eighteen foxes vaccinated orally with $10^{8.0}$ FFU, were sampled in groups of 2 at 2hrs, 1, 2, 3, 4, 8, 12, 16, and 20 days post vaccine instillation. The vaccine strain was detected in the tonsils of 7 out of 8 and in the oral mucosal membrane in 3 out of 8 animals up to the 4th day. No virus was isolated from any other sample.

Excretion of SAD B19 in the saliva of dogs

Viral excretion of SAD B19 in saliva was investigated in further experiments 2, 24, 48 and 72 hrs after oral administration of $10^{8.6}$ FFU to 11 puppies (aged 10-12 weeks) and of $10^{7.6}$ FFU to 6 adult dogs. In addition saliva swabs were taken from 8 adult dogs 1, 2, 4 and 8 days after oral administration of $10^{8.3}$ FFU. Furthermore 6 puppies were inoculated by the intramuscular route with $10^{8.0}$ FFU and saliva sampled 4, 14, and 19 days post vaccine administration. No virus could be isolated in saliva by FAT in BSR cells and/or MIT in any of the dogs or puppies under test. The sensitivity of the detection method was 100 FFU for MIT and 1 FFU per 0.5 ml of saliva in BSR cells

Efficacy of SAD B19 given by oral instillation to indigenous Turkish dogs and immunogenicity via a bait

Each of 106 owned and free-roaming dogs received 1.5×10^8 FFU of vaccine by direct instillation into the oral cavity. Serum samples were collected from the dogs which could be relocated. Detectable levels (≥ 0.5 IU/ml by RFFIT) of VNA were found in 92% (70/78), 83% (29/35) and 81% (17/21) of the dogs on average 22, 163 and 400 days after vaccination respectively. Nine out of 10 dogs captured and brought to the laboratory survived a challenge with $10^{5.3}$ MICLD50 of a street rabies virus strain (CVS/USATx Coyote/295/R/061893) 479 days after vaccination. All vaccinated dogs had detectable VNA titres at the time of challenge. All unvaccinated control dogs (6) succumbed to challenge.

Eleven of seventeen dogs which received each a Köfte bait containing 1.5×10^8 FFU of SAD B19 were relocated and bled 22 days after vaccination. Nine (82%) had VNA titres (≥ 0.5 IU/ml) on that day but no antibody could be detected thereafter (0/5 on day 163 and 0/2 on day 400 respectively). Dog vaccinated via a bait were not challenged.

Residual pathogenicity of SAD B19 in wild rodents

82 wild rodents (*A. sylvaticus*, *A. agrarius*, *M. musculus*, *M. epiroticus*, *O. zibethica*, *R. norvegicus*) received 0.1 ml of SAD B19 by the oral route at field concentration ($10^{5.1}$ to $10^{6.6}$ FFU) with the exception of *O. zibethica* which received $10^{8.0}$ FFU. During the observation period, which averaged 34 days, 8 rodents died. Five animals (3 *M. musculus*, 1 *A. sylvaticus* and 1 *M. epiroticus*) died of rabies (6.2%) while in 3 other animals (2 *M. musculus*, 1 *A. sylvaticus*) rabies was excluded as the cause of death by FAT. Untreated rodents housed in contact with those inoculated did not show any signs of rabies infection and remained negative by FAT.

Tests of SAD B19 for reversion to virulence in foxes and dogs

The genetic stability of SAD B19 was investigated in mice, foxes and dogs.

Two different experiments involving Intracerebral inoculation of 108 FFU of the vaccine virus and passaging at various time intervals were carried out.

In the first experiment 2 passages involving 3 foxes each were performed at 6 day intervals. At day 6 the 3 foxes in each passage were euthanized and 2 g of tissues were sampled from the injection site, brain stem, hippocampus, salivary glands and mandibular lymphnodes from each of these animals. Blood samples were also taken to measure VNA titres. The vaccine virus was isolated from collected tissues using both cell inoculation (BSR cells) and FAT. The sensitivity of the method was 1 FFU per 0.5 g. The experiment was repeated on 6 foxes and after 6 days the vaccine virus could not be reisolated either from injection site, brain stem, hippocampus lymphnodes and salivary glands. High VNA titres (2.5 – 34.8 IU/ml of serum) were detected in the serum of all animals.

In a second experiment 4 intracerebral passages involving 2 foxes each were performed at 2 day intervals. The animals were euthanized on the second day after inoculation and the samples were taken as described in the first experiment. A brain homogenate

containing 107.4 FFU/ml originating from the 4th intracerebral fox passage was inoculated Intracerebrally to 4 more foxes and 4 dogs (4 months old). Virus isolation was attempted from the saliva of these animals one hour and on day 1, 3, 5, 8 and 16 after inoculation. Neurological signs were investigated during the two month which followed inoculation. Blood samples were drawn on day 6 and 22 days post inoculation. Vaccine virus was not detected in any of the saliva swabs collected 1 hour and 1, 2, 3, 4 and 16 days after inoculation. All animals remained healthy and at the end of the test no virus could be detected in the brain FAT.

SAD B19 was also passaged by the intracerebral route in laboratory mice. Initially, 4 mice were inoculated intracerebrally with 0.025 ml of a SAD B19 containing $10^{8.0}$ FFU/ml. Subsequent passages were carried out in groups of 4 mice using for each new passage a 10% brain homogenate from a mouse that died of rabies in the previous passage. Rabies virus titers in the following 9 passages varied from $10^{3.5}$ FFU/ml to $10^{5.9}$ FFU/ml of brain homogenate. The 10th passage with a titer of $10^{5.2}$ FFU/ml was examined for its residual pathogenicity according to the route of inoculation. Mortality of the 10th passage in mice was 0%, 5% and 95%, by the oral, intramuscular and intracerebral routes respectively. Contact mice exposed to intracerebrally inoculated mice of all 10 passages did not seroconvert and remained healthy.

3.1.2 Live recombinant vaccines

- HAVR

No progress has been made with respect to oral dog vaccination since the report made in 1995 on the four genetically engineered HAV5 rabies glycoprotein recombinant vaccines (Ad5-RG1, Ad5- E1-RG, Ad5-RG4, Ad5-RG1.3).

-VRG

No progress has been made since the last report made in 1994. This vaccine has been extensively studied for efficacy in dogs both by instillation and via bait(s) and safety in dogs and a large number of non-target species. Experiments evaluating the safety and excretion in puppies are currently underway.

3.1.3 Inactivated vaccines, transgenic plants and recombinant plant viruses

Only attenuated or recombinant vaccines have been shown to be efficacious by the oral route in dogs but inactivated immunogens or recombinant antigens have been shown to be efficacious orally in other carnivores. Transgenic plants and recombinant plant viruses have been designed for vaccine production, including antigens for oral immunization. Plant vaccine viruses have been engineered to express and assemble determinants of rabies virus N and G proteins, using antigenic alpha mosaic virus (AMV) coat protein as carrier molecules that self-assemble, after expression by a recombinant tobacco mosaic virus (TMV). Linear epitopes of rabies virus proteins (G₅ 24, NV10c) fused individually to AMV coat proteins and expressed by a TMV vector in plants, can assemble as virus particles containing multiple epitopes from different sources.

To date in preliminary experiments, laboratory rodents vaccinated with recombinant plant viruses by the parenteral or oral routes developed VNA and one of 5 previously vaccinated dogs developed an anamnestic response to a single 100 µg dose of recombinant plant virus expressed protein.

3.2 Bait acceptability testing

3.2.1 Indonesia

A manufactured bait used in Germany for oral immunization of foxes (0,7 million baits distributed so far) was tested for acceptability in owned and free-roaming possibly ownerless dogs in a rural area and a suburban area of Sumatra, Indonesia. The bait is composed of a cylindrical envelope (biscuit-type texture) and vaccine container in this case filled with 2 ml of methylene-blue. Overall 48 baits were distributed to 45 dogs (24 owned) and the following variables recorded: time, place, dog description, distance, duration until contact/acceptance, intensity/type of contact with the bait/container, amount of bait substance consumed, vaccine container fate, penetration, marker and vaccine spillage/loss.

Baits, when accepted, were always fully consumed both in owned and free roaming/ownerless dogs (11secs average time between presentation and bait contact). Baits and their “vaccine”containers were quickly taken up and most were rapidly and fully consumed by owned dogs (baits: 87 % completely consumed; container: 65% punctured, 30 chewed and swallowed). Only one third of the baits presented to free roaming /ownerless dogs were fully consumed. In these instances 71% of the containers were penetrated and 14% intensively chewed and swallowed. Bait handling was easy due to its dry texture and inoffensive odour.

3.2.2 Thailand

A bait acceptability study in Thai dogs was conducted in the Nakornsawan province. Four groups of dogs were studied: household dogs (owned dogs), temple dogs (owned and community dogs), market dogs (community dogs), street dogs (unowned dogs). Each group of dogs was offered 5 kinds of baits, consisting of a locally produced dog chewing bone (a flat, hard rectangular -3x5x2cm- bait), flavored with liver aroma, fish aroma, chicken aroma, natural aroma, or a commercial fish-flavored, hollow bait used to deliver VRG to foxes in Europe. No vaccine was placed inside the bait.

In both rural and urban communities, the liver-flavoured and commercial baits were taken more often by all dog categories. Overall bait acceptance was highest in owned and temple dogs and lowest in market dogs. Of all dog categories together local baits with a liver-flavor and commercial VRG baits were taken more often than the other baits and this in both rural and urban environments.

Bait acceptance rates in rural and urban areas, based on the time of day, were as follows;

Time	Urban	Rural
06.00-09.00h	53%	52%
10.00-15.00h	40%	61%
After 1600h	57%	59%

3.2.3 Sri Lanka

A bait acceptance trial was conducted in 10 randomly selected areas of approximately 3000 inhabitants each, situated 30 km north east of Colombo in Sri Lanka. Prior to the bait acceptance trial, a door-to-door parenteral mass vaccination campaign was carried out and vaccinated dogs were identified by a collar. Immediately after, 800 baits without VRG vaccine were distributed by volunteers to unmarked dogs and observations on bait consumption were recorded. Of 571 baits distributed, 212 were offered to free-roaming dogs (37%) and 359 to unmarked dogs found in homes (62.9%). Of the latter, 82% accepted a bait (n=174), compared to 86% in free roaming dogs (n=309). The average time taken for consumption of the bait was 6.9 minutes. The baits were swallowed by 2.8% house compared to 5.7% of free-roaming dogs. Bait acceptance was not statistically different between males and females (P=0.1), between juvenile or adult dogs (P=0.08) or between different localities.

3.3 Testing of bait distribution methods in Turkey

A survey carried out in the town of Kusadi, 95 km South of Izmir had shown that 297 of 4701 households visited owned at least one dog. Of the 481 dogs counted, only about 20% were shown to be vaccinated against rabies during the previous year and, in view of the lack of evidence, it had to be assumed that about 40 % of them were not vaccinated. Forty-two and twenty-nine percent respectively of 423 dogs were reported to be always or sometimes restricted. In April 1997, a door-to-door vaccination campaign was carried out in this town following a mass media information campaign. Dog owning households were visited by vaccination teams. Accessible dogs (owned and ownerless) were vaccinated against rabies by the parenteral route, while inaccessible dogs were offered a Koefte SAD B19 bait. A total of 189 dogs (73.3%) were vaccinated parenterally and 69 (26.7%) orally. Oral vaccines were offered by the vaccination team to 84 dogs. Two dogs fled when approached and 13 did not puncture the vaccine capsule or swallowed it intact. Of 117 unrestricted dogs encountered, 69 (59%) were offered a bait which was in most cases accepted. Unrestricted dogs were vaccinated by the oral route significantly more often than restricted dogs. No contact of humans with baits and vaccine capsules was observed since if not taken by the dog, they were immediately collected by the vaccination team.

In a second study, 150 Koefte baits containing the biomarker Clenbuterol (0.42-1 mg/bait) and a capsule with SAD B19 at a concentration of $1.5 \times 10^{8.0}$ FFU/ml were placed at selected sites in urban area of Istanbul overnight. The next morning, 93% of baits had disappeared. Five to 6 weeks after bait placement, 30 free-roaming, owned and ownerless dogs, from which a seronegative blood sample had been obtained before bait distribution were recaptured. Serum samples obtained from these dogs were analyzed by RFFIT and hair

samples by Clenbuterol-enzyme immunoassay. Sixteen of 30 dogs did not have detectable levels of either antibodies or Clenbuterol, indicating that over 50% of the recaptured dog population had not consumed a bait. Of the remaining 14 dogs, 6 (20%) exhibited VNA titers above 0.5IU and 9 (30%) had significant Clenbuterol levels. Blood and hair samples from one dog were both positive for VNA and Clenbuterol respectively. The high bait disappearance rate suggests important non-target interference. The low vaccination and bait consumption rates in free-roaming dogs may indicate that overnight placing of baits is not a very efficient method to vaccinate ownerless dogs. On the other hand, these results may result from bait rejection or swallowing of intact vaccine capsules or failure to ingest Clenbuterol because of localized distribution of the marker in the bait.

4. ESTIMATING DOG POPULATION SIZE BY A BAYESIAN MARK-RECAPTURE MODEL

For evaluating rabies control programs and assessing the potential use of new vaccination strategies, methods to precisely estimate the size of dog populations and the accessibility of dogs to specific control measures are needed.

To study the size and structure of different population segments in a limited area of Sri Lanka, a combination of different techniques including household surveys, collar marking, and recapture/reobservation of dogs was used. A Bayesian capture-recapture model which allows recapture probabilities to vary between different population segments was developed. This is important because catchability of marked and unmarked dogs in a population - a sensitive assumption of the Petersen-Lincoln estimator recommended by WHO - is often variable. The model assumptions included a closed population, no marker loss, no intra-group recapture variability, and no exchange of individuals between groups. The dog population was stratified by age (puppies, adult and juvenile dogs), collar marking after a one-day-vaccination campaign (marked dogs, unmarked dogs), confinement (free-roaming, not free-roaming), and ownership status (reference household identified, no reference household identified). Recapture probabilities were defined as the product of:

- the probability that a given area is covered during a recapture passage;
- the conditional probability that a specific dog is encountered given that its present location is within the area covered by recapture;
- the conditional probability that a dog is «visible» from the recapture line given that its present location is within the recapture area and that it has actually been encountered and,
- the conditional probability that a dog within the recapture area was actually recorded given that it was encountered and «visible» from the recapture line.

Prior information on these four components was available for puppies, confined dogs (juveniles and adults), and free-roaming dogs (juveniles and adults). Specification of “priors” for the different population segments included in the model were based on household survey data, local expert knowledge, and experiences acquired from similar studies in other countries. The posterior distributions of all model parameters were derived by the Markov Chain Monte Carlo methodology, and their mean and 95% credibility intervals were calculated. With the

Bayesian methodology, prior knowledge on all parameters can be incorporated in a flexible way and subpopulation sizes and recapture probabilities can simultaneously be estimated, which is an essentially undefined problem in the classical approach. This model may be adapted to most situations where it is possible to mark dogs and to carry out a household survey. The complexity of the model and the need for a powerful computer may however limit a more widespread use of the present model. WHO may provide assistance if the use of this method is contemplated for the description of dog populations and evaluation of oral dog vaccination field trials.

5. OUTLINE OF PROPOSED ORAL VACCINATION FIELD TRIALS

5.1 Mexico

A protocol for a trial has been submitted for evaluation which considers vaccination of dogs by the oral route with the SAG2-DBL2 vaccine bait in the town of Atlixco Puebla, Mexico. This town was the site of a previous trial in which bait acceptability was tested in selected households with dogs. The population of the town is familiar with bait-acceptance trials. The trial will include 3 neighbourhoods. Only household dogs of both sexes, 2 months or older and clinically healthy will be included in the study.

Seven hundred and fifty animals will receive an oral vaccine, consumption observed and leftovers picked up. The protocol recommends that human contact with freshly vaccinated dogs be avoided for 1 to 2 hours. Serum samples will be collected at day 0 and 40 after OV. Neutralizing antibodies (VNA) and antinucleoprotein antibodies will be measured as an indicator of immunogenicity and adverse reactions monitored. Other data to be collected should include: dog-human ratio, use of the dogs and family dog environment

5.2 South Africa

South Africa hopes to conduct field trials using SAG2 before the end of 1998. DBL2 baits containing SAG2 vaccine will be distributed on a house-to-house basis and a dog census carried out simultaneously in two selected areas of Kwazulu-Natal. Acceptability of the baits by dogs and by the communities will be assessed and the information obtained from the dog census will allow estimates of bait requirements for the affected areas to be made. South Africa has set an objective of the elimination of rabies by the year 2010 and the oral vaccination of dogs forms an integral part of those long-term plans.

5.3 Thailand

The rabies unit of the Division of Disease Control, Department of Livestock, Ministry of Agriculture plans to conduct a study using the SAG2-DBL2 vaccine bait in one of the rabies infected districts of the Kanchanaburi Province (125km west of Bangkok). Rabies cases in both animals (mainly dogs) and humans have been reported each year from this area during the period of 1995-97. The study should aim at:

- measuring the immunogenicity of one SAG2-DBL2 vaccine-bait in dogs with different ownership status (owned, semi-owned and ownerless) over a period of one year;
- assessing bait acceptability according to dog status;
- evaluating the efficacy of the technique through measuring its impact on rabies incidence in the area.

The Department of Communicable Disease Control, Division of General Communicable Diseases in the Ministry of Health is also developing a protocol for the study of the acceptability and immunogenicity of the VRG bait in the Nakhorn Sawan Province (250 km north-west of Bangkok). The study should involve two villages. In addition to evaluating bait acceptability and vaccine immunogenicity with one bait, the study design should allow for an estimation of the difference in the vaccination coverages achieved with a combination of oral and parenteral vaccination versus parenteral vaccination alone.

5.4 Indonesia

Oral immunization of dogs is seen as a valuable complement to parenteral dog immunization in areas of Indonesia particularly in West Sumatra and Kalimantan. In these areas dog owners, particularly of hunting dogs, do not bring their animals for vaccination. As reported earlier, misconceptions about side effects of parenteral immunization as well as cultural or religious beliefs may be factors limiting dog immunization. The study design remains to be written. Considering the situation apparently prevailing in the area the study should aim at immunizing owned dogs and should combine parenteral and oral vaccination, particularly of hunting dogs.

5.5 Sri Lanka

A project combining parenteral and oral vaccination of dogs is proposed in Pandura-district. Following a rabies information campaign, blood samples will be collected in an area with approximately 27,000 dogs. A parenteral vaccination campaign will be conducted, during which vaccinated dogs are marked by collars. Subsequently, unmarked dogs will be offered a VRG bait. The vaccination coverage established by the parenteral vaccination campaign will be assessed using a capture-recapture technique. Blood samples from a representative sample of orally vaccinated dogs should be tested for rabies VNA titres. The study will also include activities to actively search for any adverse effects in animals and humans that may result from the oral rabies vaccine.

6. FINAL CONCLUSIONS AND RECOMMENDATIONS

Increasing the vaccination coverage in dogs considered inaccessible to parenteral vaccination by making use of OVD, may lead to establishing a level of herd immunity high enough to eliminate canine rabies in a given dog population. Although progress has been reported concerning vaccine and bait development as well as placebo bait distribution methods, little is known about the field application of vaccine baits in the context of dog rabies control.

For the time being, the use of vaccine loaded baits for the oral immunization of dogs is considered experimental and their distribution should initially be limited to controlled field trials conducted according to protocols allowing extensive monitoring of the fate of vaccine baits. The results of a trial should be investigated in terms of:

- target species as well as human and non-target species exposure to the vaccine;
- bait acceptance and vaccination coverage attained in the dog population.

It was recognized that, in view of the variety of vaccines involved and the insufficient number of data on excretion in dogs of the various input vaccine viruses (live modified and recombinant vaccines) currently available, no valid generic recommendation on the duration of dog restriction after oral vaccination should be made, and complementary excretion studies involving larger number of dogs with swabbing at regular and frequent intervals after vaccine instillation should be carried out. Sensitive techniques for virus isolation should be used in such studies. During the field trials, advice aiming at avoiding contacts between freshly vaccinated dogs and humans to minimize risks of vaccine virus transmission should be based on the results of a study carried out as described above with the vaccine under trial.

Several countries are presently volunteering to pioneer the oral vaccination of dog technology in their territories in collaboration with vaccine producers, scientists and WHO. As more information about the field application of OVD should become available soon, WHO should continue to provide up-dated guidance for the safe and cost-effective use of OVD to field researchers as well as public health and veterinary officers in charge of national rabies control programmes.

The Consultation was expected to review, amend and possibly endorse a document entitled "Guidelines for research on oral rabies vaccines and field application of oral vaccination of dogs against rabies", prepared as a draft prior to the meeting under WHO secretariat guidance. However time did not permit to review the entire document in details and it was agreed that comments from the participants should be sent to the WHO secretariat by the end of November 1998 for consideration and possible inclusion.

During this meeting, the group concentrated on defining criteria for country and site selection and identified indicators of success for field trial evaluation.

6.1 Qualification criteria for a country contemplating the use of OVD

These criteria are:

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

In addition:

- An evaluation of the programme of parenteral vaccination of dogs suggests that OVD may improve the vaccination coverage;
- The OVD trials are part of the operational research component of the national programme for rabies control;
- There are national or international collaborations in place with organizations such as WHO and its reference laboratories able to assess the trial protocol and its implementation, to identify rabies virus variants, to perform serology and additional modern techniques - as detailed in "Laboratory Techniques in Rabies", Fourth Edition, WHO, Geneva, 1996.

Protocols for OVD protocols and trials should receive the approval of all regulatory and other national authorities concerned. The OVD trial plan should include the:

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

6.2 Field trial site selection and characteristics:

The area should:

- have a current history of documented rabies in dogs;
- be representative of potential target areas;
- be geographically well defined - but not necessarily isolated. A priority is that it must be accessible for campaign and follow-up;
- be large enough to allow the recruitment of a statistically significant sample of dogs allowing the evaluation of:
 - vaccination coverage (Section 6.3);
 - time, manpower and logistics required for vaccinating dogs per oral or parenteral route;
 - manpower efficiency;
 - relative cost of various dog immunization strategies: OVD alone, parenteral vaccination alone, combination of OVD and parenteral vaccination,
 - safety of the method for non-target species and level of human contacts with bait and exposure to vaccine
 - have a foreseeable active support of the community.

6.3 Indicators of success

The group felt that success of a vaccination campaign could be measured in terms of efficacy and safety.

In terms of efficacy as a primary indicator of success of an oral vaccination campaign in dogs, vaccination coverage should be assessed. Observational data should be collected allowing the evaluation of as many of the following parameters as possible:

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

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

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

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

6.4 Standard protocol for field trials

At this point, the consultation considered, too little information was available to propose a standard protocol for OVD field trials, but it may be useful if future prospective protocols were discussed in details with WHO.

6.5 Assessment of candidate vaccines compliance with WHO recommendations

To assess candidate vaccines and their current state of compliance with WHO recommendations and until the “Guidelines for oral rabies vaccines and field application of oral vaccination of dogs against rabies” are completed, the WHO secretariat would like to refer national authorities or Ad-Hoc national committees, to the efficacy and safety requirements established by the series of WHO Consultations on Oral Immunization of Dogs (reports available from WHO). The WHO secretariat wishes to reiterate that the assessment of the level of compliance of an oral vaccine for dogs should be made on the basis of the results of the experiments carried out with that vaccine and its bait (independently and in combination) under laboratory and field conditions. Special attention should be paid to data on vaccine efficacy in dogs via a bait, safety for non-target animal species and other tests (e.g. inoculation to non-human primates, virus excretion in target species), as well as distribution methods to characterize risk for humans. WHO is ready to assist in this assessment if requested.