



REPORT OF
WHO/APHIS CONSULTATION ON BAITS AND BAITING DELIVERY SYSTEMS
FOR ORAL IMMUNIZATION OF WILDLIFE AGAINST RABIES
Colorado State University, Fort Collins, Colorado, 10-12 July 1990

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1. INTRODUCTION

The convening of the Consultation was prompted by several considerations:

- the fact that the first field trial for assessing the safety of oral immunization of wildlife in the USA will take place very soon. This will, hopefully, open the door for larger field experiments for evaluating the efficacy of the method for raccoon rabies control and subsequently for controlling the disease in the USA;
- the need to develop specific baits and delivery systems for the various species transmitting rabies in the United States (skunks, mongooses and red and Arctic foxes);
- the need for new concepts in bait composition and bait delivery to elaborate the most cost-effective strategies for the control of wildlife rabies in the Americas and also in Europe;
- the need for further national and international cooperation in this field, which can only be achieved by bringing together experts in rabies epidemiology, wildlife management, attractants and flavours development, bait preparation, etc.

The objectives of this meeting were therefore to propose lines for further research on bait composition (including attractants and biomarkers), their evaluation, and bait delivery systems, with special reference to the ecology of each major rabies host species present in the Americas.

The participants (see Annex 1) were welcomed to Fort Collins by Dr S. Linhart on behalf of the Animal and Plant Health Inspection Service of the United States Department of Agriculture (APHIS) and by the Director of the Denver Wildlife Research Center (DWRC). On behalf of the Director-General of WHO, Dr F.-X. Meslin welcomed the participants and thanked APHIS which had co-sponsored the Consultation, DWRC which had accepted the major burden of local organization, and the Colorado State University which had hosted the participants. Dr S. Linhart was elected Chairman and Dr E.H. Follman was nominated Rapporteur.

2. ONGOING FIELD AND RESEARCH PROJECTS FOR ORAL IMMUNIZATION OF FOXES

2.1 France

Two new baits (Dupont-Mérieux and Virbac baits) were tested to evaluate their acceptability, shock-resistance and uptake rates. Acceptance trials were performed on caged foxes. After two days, 74% of the Virbac baits (33 foxes) and 82% of the Dupont-Mérieux baits (28 foxes) had been eaten.

The Virbac bait is a brown bait made of paraffin, fat and fishmeal and is similar in shape and size to the German Tübingen bait (dimensions 4.5 x 4 x 1.5cm, weight 25g). The tetracycline used as a biomarker is mixed at a concentration of 150mg/bait. The vaccine container is a thermo-formed PVC shell (24mm diameter, 6mm high) sealed with an aluminium sheet. When submitted to high and low temperatures and different rates of moisture it showed good mechanical shock-resistance and attractiveness for foxes, the bait's structure remained unaltered and the vaccine container remained enclosed in the bait matrix.

When 10 frozen baits of each type were dropped from a helicopter, all the Dupont-Mérieux, 6 Virbac and 4 Tübingen baits were undamaged.

Uptake rates by species were estimated by using track stations on which 13 Virbac, 12 Mérieux and 13 chicken head baits were deposited manually. After 7 days, the overall bait uptake rate was 94.7% (87.5% for birds, 9.3% for foxes and 3.2% for stone martens).

Hand distribution of 105 Dupont-Mérieux and 104 Virbac baits was carried out in two areas. Every bait's location was recorded on a map. The bait uptake rate was measured 6, 7 and 12 days later. The numerator used for calculating the final bait uptake was the number of baits which disappeared, plus those eaten whose vaccine container was retrieved with punctures.

For the Dupont-Mérieux bait and for the Virbac bait, the uptake rates were 80.4% on Day 6, and 88.5% on Day 12, and 87.8% on Day 7, and 89.8% on Day 12 respectively.

The aerial bait distribution of rabies vaccines is carried out in spring and autumn using two helicopters. The team comprises two pilots, one mechanic and at least four bait droppers. This team can treat between 1 200 and 1 700 km²/day and should cover 63 000 km² in autumn 1990.

An average of 15 baits/km² is distributed. Control after baiting is performed by tetracycline analysis and titration of rabies antibody in fox carcasses. On 247 samples (teeth or bone), 152 were tetracycline positive (62%). After a first vaccination campaign, the seroconversion rate was 42%. After a second campaign in the same area 62% of the foxes had satisfactory antibody titre (i.e. > 0.3 IU/ml).

2.2 Other European countries

Five different types of baits are used in Europe:

- (a) Chicken heads in Switzerland,
- (b) The "Tübingen fox bait" in the Federal Republic of Germany, as well as in most other western and eastern European countries where oral vaccination projects are underway (Belgium, Luxemburg, France, Italy, Austria, Yugoslavia, and Czechoslovakia),
- (c) the fishmeal polymer (FMP) bait from Mérieux, used in limited areas in Belgium and France during the period 1988-1989 and more widely in France in 1990 (more than 200,000 baits distributed),
- (d) The "Virbac" bait which has been distributed over more than 10 000km² in France (200 000 baits used in 1990),
- (e) The East German bait.

Individual bait characteristics and distribution strategies in Europe for the period 1978-1988 are shown in Annex 2.

Manual bait distribution is the most common method in Europe. Baits are placed at sites which have been predetermined and marked on a map. This technique permits a uniform distribution of the baits over the area. Bait placement is done by various professional and nonprofessional groups (game wardens, auxiliary game wardens, police and hunters). In Germany and Switzerland manual bait placement is facilitated by the organization of the hunting system (refer to article by V. Wilhelm and L.G. Schneider in WHO Bulletin 68 [1] 87-92 [1990]). In Belgium and France, where hunting is

differently organized, the cooperation of hunting societies is more difficult to secure. In these countries, it has therefore been decided to switch to aerial distribution using small helicopters (see 2.1).

A density of 15 baits/km² is a standard for oral vaccination of foxes in all European countries involved in such activities. Data bait densities, bait acceptance rates and percentages of foxes found positive for tetracycline per country and bait type are presented in Annex 3.

Areas where campaigns for oral immunization of foxes were carried out during the period 1978-1988 and also during 1989 are shown in Annex 4.

2.3 Canada

Since 1980 the Ontario Ministry of Natural Resources, Wildlife Research Section has been developing an aerial baiting programme capable of large-scale distribution of rabies vaccine baits. The primary target of the programme is the red fox (*Vulpes vulpes*). Ten years of experimentation has resulted in the development of a mass-produced (20 000/week) bait consisting of a plastic blister pack containing ERA-BHK₂₁ cell line vaccine, enclosed in a wax tallow cube containing artificial chicken essence attractant and a tetracycline biomarker. Equipment installed in a de Havilland Twin Otter aircraft is capable of delivering baits at densities of 20 baits/flight km, at ground speeds of up to 300 km/h. The first large scale field experiment was undertaken in October 1989. Two large areas of southeastern Ontario were baited: the "West Wall" zone (178 924 baits distributed over 9 200km²) and the "Peninsula" zone (98 395 baits distributed over 5 000km²).

The Loran-C navigation aid used in Ontario, Canada, for aerial distribution of vaccine baits provides a means of following flight lines and determining the aircraft ground speed. Latitude and longitude of flight line waypoints can be read from topographic maps and entered into the memory of the Loran-C. The Loran-C displays ground speed which must be known so that the correct bait dropping rate can be set to achieve the desired bait density (baits/km²) on the ground. Flight lines can be drawn to cover optimum habitat and avoid unbaitable areas such as towns, water bodies etc.).

The flight line spacing required to bait a particular species varies inversely with the size of the animal's home range. Red fox and coyote can be baited with flight lines spaced 2 km apart, whereas skunks and raccoons require close spacing depending on the pattern flown. Bait densities required to achieve significant levels of bait acceptance vary with species and habitat. For example, the Ontario aerial bait distribution trials have shown that a rate of approximately 23 baits/km² can achieve an acceptance rate as high as 74% in red fox.

The best season for aerial distribution of baits depends on the country and the animal species being treated. With wild carnivores in the northern hemisphere, activity is concentrated around whelping dens in spring. The young are small and may not find baits unless they are brought to them by adults. They may also not respond well to the oral vaccine. Baits dropped during summer may be prone to heat inactivation, insect fouling and rodent depredation. According to the Canadian experience, autumn or early winter is the best time for baiting. Juvenile animals are expanding their movement and will encounter baits, and they are also fully immunocompetent.

Bait performance, in terms of seroconversion rates of foxes eating baits, has been investigated in both laboratory and field trials. Caged foxes fed baits under controlled laboratory conditions consistently had seroconversion rates higher than animals encountering baits in field trials. Caged foxes who were fed baits had seroconversion rates of 77-85%. In comparison, wild foxes collected during field trials and known to have eaten at least one bait, had seroconversion rates of 32-53%.

Several possible explanations for this phenomenon were considered. Namely:

- (1) Baits subjected to environmental conditions in the field lose vaccine titre and become less effective in producing seroconversion in foxes;
- (2) Foxes eating baits "spill" some vaccine thus reducing the chance of seroconversion;
- (3) Samples taken from animals in field trials are contaminated and reduce or invalidate the results of ELISA blood tests; and
- (4) Foxes encountering baits can eat the outer tallow shell and avoid the vaccine packet, resulting in "false negatives" in the results.

3. CURRENT AND FUTURE RESEARCH PROJECTS ON BAIT DEVELOPMENT AND EVALUATION

3.1 Acceptance of cod-oil and chicken-essence baits by skunks and raccoons

Since oral immunization with vaccine-baits is the most feasible approach to rabies control in skunks and raccoons, attempts to develop a bait and attractant combination that will achieve maximum acceptance by those two species were made. During 1989, two types of baits were field tested:

- (1) Chicken essence wax tallow,
- (2) Cod-oil-wax tallow in the urban skunk rabies vaccination study area in metropolitan Toronto. Tetracycline hydrochloride (100mg/bait) was incorporated in the matrix of the bait to serve as a biomarker.

Baits were distributed in field or forest-park habitat in each of sixteen 1 km^2 cells within the 60 km^2 vaccination area between June and October 1989. The density of baits varied between 25 and $147/0.04 \text{ km}^2$ of field or forest-park habitat in each 1 km^2 cell. Each cell was investigated one week after baiting and teeth were extracted from live-trapped skunks and raccoons to determine bait acceptance.

Of the 1211 baits distributed, 612 were cod-oil and 599 were chicken-essence baits. A positive correlation was found between bait density and bait-acceptance for skunks and raccoons with the cod-oil bait. Also, cod-oil bait-acceptance by raccoons was greater than for chicken-essence baits. Acceptance by raccoons at a density of 147 baits/ 0.04 km^2 of forest park was about 68%. Raccoon density in that area was higher than $50/ \text{km}^2$. Cod-oil bait acceptance for skunks was 38% in areas that were baited at a density of 99 baits/ 0.04 km^2 of field in each 1 km^2 cell. Those areas had skunk densities higher than $20/ \text{km}^2$.

In order to evaluate vaccinal bait efficacy, it is planned to incorporate tetracycline in the blister-packs of baits during 1990 and to repeat the 1989 experiment at a density of 175 baits/ 0.04 km^2 of field or forest-park habitat per 1 km^2 cell.

3.2 Advances in raccoon bait composition and design

With the advent of safe, efficacious oral wildlife rabies vaccines, baits and delivery systems originally designed for the European red fox are being modified and refined for a variety of wildlife rabies vectors, including the North American raccoon. Since 1985, baits designed for the European red fox have been utilized in a variety of raccoon baiting strategies with a moderate degree of success. However, innate differences between the species with respect to attraction to the bait, bait

manipulation, consumption and penetration of the vaccine chamber, have led to the development in the Wistar Institute of Anatomy and Biology in Philadelphia, of a unique bait designed for raccoons.

The fishmeal polymer bait has been evaluated in a variety of experimental designs including captive raccoon trials, smorgasbord and paired, direct observation trials on free-ranging raccoons, and placebo baiting trials. A proven biomarker, tetracycline, has been incorporated in the bait matrix with no apparent decrease in bait acceptance by raccoons. During placebo baiting trials, raccoon bait acceptance based on this reliable postmortem biomarker has ranged from 65 to 100% depending upon bait density, size of the study site, and the number of raccoons harvested. Evaluation of bait acceptance based upon currently available ante-mortem biomarkers has been less reliable and has led to further investigation of a variety of candidates. These have included ante-mortem analysis of tetracycline, rhodamine B, fluorescein yellow, iophenoxic acid, sulphamethazine and sulphadimethoxine. The requirements of a successful biomarker include:

- (1) safety and efficacy in target and non-target species;
- (2) affordability;
- (3) minimally invasive sample collection;
- (4) economical and readily performed analysis;
- (5) palatable formulations readily available or easily made;
- (6) flexibility allowing incorporation in the vaccine chamber, bait matrix, or attractant slurry for efficient testing of each of these components; and
- (7) of sufficient duration to permit effective assessment.

Based on the above criteria, promising ante-mortem biomarkers include sulphamethazine and sulphadimethoxine.

3.3 Baiting trials on caged and free-ranging raccoons

The following trials were carried out by the Denver Wildlife Research Center, in collaboration with Centers for Disease Control, Atlanta, and the Georgia Department of Natural Resources.

In 1988, captive raccoons were offered a variety of vaccine containers and bait components in a series of three-choice tests. Paraffin bait components included corn and shellfish oils, deep-fried corn meal batter, and egg, apple and buttermilk flavourings. These results, together with factors including ease of bait formulation, cost and suitability for field use, were used to develop an experimental delivery system for an oral rabies vaccine.

This system was composed of a polyurethane sleeve (1.5 x 5.5 cm) dipped in a commercial food batter mixed together with corn meal, milk and egg. The sleeve was deep fried in corn oil and a 2.0 ml ampoule containing a recombinant rabies vaccine was then inserted into the sleeve bait. These baits were presented to 10 captive raccoons. Nine of the 10 animals developed high levels of rabies virus neutralizing antibodies.

Four field trials were conducted in 1989 in Georgia and Maryland to evaluate raccoon baits and baiting strategies for delivering oral rabies vaccines. A deep-fried, food batter-based bait was well accepted by wild raccoons. Raccoons visited a high percentage of bait stations (32.2 - 44.3%) and took 79.0 - 89.9% of the bait packets within 4-5 days. Less than 1.0% of all baits taken were found partially

eaten and no rejection of water-filled paraffin ampoules in baits was observed. The use of an odour attractant on bait packets did not appear to enhance bait discovery when packets were placed on raccoon travel routes, but did enhance discovery when baits were placed off-road in a simulated aerial bait drop test. Non-target species often visited stations (31.2 - 53% of all visits) and took bait packets (28.3 - 54.9%) but did not appear to seriously affect the availability of baits for raccoons.

In May 1990, 2 300 raccoon baits containing iophenoxic acid were placed out on 28 km² (= 82 baits/km²) on Sapelo Island, Georgia, to determine the percentage of the raccoon population that could be reached by baits. A check of 100 baits placed on tracking stations indicated that 84% were taken in two nights of exposure. Raccoons took 52% of the baits and visited 46 stations where they took 96% of the baits. Non-target species took 36% of the baits. Twenty-four raccoons were live-trapped before baiting to obtain baseline blood iodine levels; 54 raccoons were trapped and bled following bait distribution. Results of the above field test are not yet available.

3.4 A baiting system for mongooses

Mongooses are important rabies reservoirs in many parts of the tropics, including four Caribbean islands. Immunization with an oral vaccine appears to be the only feasible approach for rabies control in mongoose populations. Bait delivery systems for oral vaccines were developed and tested. Baits tested included egg flavoured sponge, tallow wax chicken, tallow wax beef, tallow wax fish, manioc meal balls, corn meal balls, hot dog sausage, cowhide gelatin, hard-boiled egg, soft-boiled egg, fish flavoured sponge, and fishmeal polymer. The baits were tested fresh, and after 5 and 10 days' incubation at 34°C in a high humidity chamber, to simulate tropical field conditions. Candidate vaccine containers were also tested with several of the baits, including a paraffin ampoule, polyethylene capsule, plastic sachet, and plastic wrap-covered sponge. The egg-flavoured sponge bait with the paraffin wax container provided the best combination for mongoose preference, stability over the 10 day test period, and simulated vaccine exposure in caged mongooses, as well as low cost and logistical simplicity.

Odour lures were tested in the fields as mongoose attractants. Lures tested included Synthetic fermented egg (SFE), Fatty Acid Scent (FAS), Trimethylammonium decanoate (TMAD), TMAD plus sulphides (W-U), Mongoose anal gland acids, Carman's canine call, and an organic fish compound. Attractants were tested using tracking stations. The design permitted the simultaneous testing of the seven mentioned odour lures and a control. Preliminary field trials of the egg-flavoured sponge bait were performed.

3.5 Specific problems and possible baiting strategies for Arctic rabies control

Development of baiting strategies to immunize Arctic foxes (*Alopex lagopus*) against rabies will be necessary for an effective control programme. No baiting trials have been conducted in the field in Alaska to date either to determine the effectiveness of baits or to attempt immunization of foxes. Laboratory experiments have included the feeding of sausage baits containing the blood marker iophenoxic acid and the SAD-BHK₂₁ rabies vaccine to foxes, testing of the flavoured tallow baits used for red foxes in Canada to determine whether they would be suitable for Arctic foxes, and feeding trials of the tallow and beeswax baits used by the U.S. Fish and Wildlife Service in the fox control programme on Kiska Island, Alaska.

Although there has been considerable field experience with bait vaccine programmes in Europe and in Canada, high latitude regions pose unique problems. Factors to be considered are the seasonal presence of sea ice, ambient air temperatures, and the terrain as well as the ecology and behaviour of Arctic foxes as biotic factors that could enhance or limit the effectiveness of a vaccination programme.

Depending on the year and weather conditions, shore-fast sea ice can remain throughout the year in northern Alaska or at least be present from October through June; its occurrence in western Alaska is usually for a shorter period. Arctic foxes move onto the ice quite readily and can use it to move to other circumpolar land masses, thus potentially affecting the success of a vaccination programme by loss of vaccinated animals from the baited area or by an influx of unvaccinated animals from other regions. The extent of this movement is unknown.

Ambient air temperatures remain well below freezing for extended periods that can range from September through May. Given the need to maintain current oral rabies vaccines in liquid form, baiting during this period would be ineffective. During the summer in northern Alaska freezing temperatures can occur every day thus exposing vaccines to periodic and/or irregular freeze-thaw conditions which could render the vaccine ineffective for oral immunization of foxes.

The extensive land masses over which epizootics of rabies occur periodically, which are enormously enlarged when shore-fast ice joins the pack ice, provide a challenge with regard to the size of area that can be effectively protected. As a priority, relatively restricted areas surrounding villages and remote industrial sites are protected when a rabies epizootic is anticipated.

Arctic foxes in northern Alaska maintain their territory during the breeding and pup-rearing season which extends from about March to August. During the remainder of the year foxes roam about in search of food which becomes less abundant and available during winter. Their tolerance for one another increases during this time so that 20 or more foxes may be present at a large food source, such as a walrus carcass or a garbage dump. This would allow ease of baiting, but, unfortunately, freezing temperatures during this period would limit success.

During summer the dens of the Arctic foxes in flat tundra environments are often found on prominences such as pingos and river terrace banks, and are often distinguishable by the lush, green vegetation resulting from the greater nutrients there than on the surrounding tundra. The relative ease of finding active dens during the summer would enhance the success of a vaccination programme because foxes could be immunized with far fewer baits than would be necessary if a transect distribution programme were used in these areas.

4. POPULATION ECOLOGY AND OTHER STUDIES

4.1 Techniques for censusing and indexing furbearers

Density estimators applicable to furbearer populations fall into six categories:

- (1) direct counts or capture-recapture;
- (2) counts of dens, tracks, etc.;
- (3) questionnaires/cooperator surveys;
- (4) catch per unit effort;
- (5) elicited response surveys;
- (6) population modelling.

Capture, mark, release and recapture studies can generate reliable estimates of population densities in relatively small areas, provided a number of conditions are met. Various methods of capture and marking animals are available and can be selected according to species attributes.

Studies to determine the relationship of scent station index (SSI) values to furbearer densities have been conducted in the southeast. Concurrent surveys were conducted with increasing bobcat densities as part of a bobcat reintroduction programme on Cumberland Island, Georgia. A significant correlation ($r = 0.787$) was

observed between SSI values and bobcat densities. Similar correlation has been demonstrated for raccoon populations in Tennessee. Efforts to document a SSI/grey fox density relationship in North Carolina have been interrupted by a canine distemper epizootic. SSI surveys in the study area have tracked the resulting population reduction over time.

Population estimates based on capture, mark and recapture techniques can produce accurate information, but are expensive in terms of effort. SSI surveys can be used over large areas to monitor trends in furbearer populations, and with validation will likely prove efficient as predictors of absolute densities. Standardization of the methodology and calibration of the survey are major shortcomings.

4.2 Ecology and behaviour of urban raccoons

The raccoon population of Rock Creek Park, a 1754-acre natural area located entirely within the confines of metropolitan Washington, D.C., has been studied since 1983 in an effort to further understand the behaviour and ecology of this species within an urban park setting. A baseline radio-telemetry study in 1983-1984 focused on the movements and activity patterns of approximately thirty animals in the southern portion of the park. Life history studies and the collection of data on morphometric and reproductive characteristics have been performed on the more than 700 animals which have been live-trapped and examined. The live-trapping programme has utilized a mark-recapture methodology to estimate population levels at two intensive study sites. An additional study of feeding ecology was conducted in 1985.

A test of oral bait acceptance was conducted in 1986 in an 80-hectare study area adjacent to a residential neighbourhood. Finally, a study of spatial relationships between adult females and their young was conducted in 1989-1990 and involved an additional 22 radio-collared animals.

Results from these studies indicate that population densities, as estimated by mark-recapture techniques, are high compared to those derived from studies in rural areas, with an overall estimate of one raccoon for each 0.935 hectare. Studies of movement and activity patterns indicate considerable home range overlap, with animals utilizing both the park and surrounding residential areas. Animals remain active throughout the year and cease nightly movements only for relatively short periods of time in the coldest weather. The availability of food from human sources throughout the winter months may contribute to the observed trend for Rock Creek raccoons to lose relatively less of their body weight during winter than raccoons in more rural habitats. Communal denning was frequently observed among adult males in the 1983-84 study and was less frequent among adult females during the 1989-90 study. The timing of the dissolution of familial bonds is highly varied, with some mothers associating with young throughout the first winter following birth and others separating much before that. Sibling associations and contacts may persist after separation from the mother. Rabies is currently enzootic in the raccoon population in Washington and, together with canine distemper, represents a major disease-related source of mortality.

4.3 Population ecology of striped skunks

There are 13 species of skunks in the New World including striped, spotted, hooded and hog-nosed skunks. However, as the striped skunk accounts for the majority of rabies diagnosis in skunks, this account is limited to that species. Striped skunks are found throughout southern Canada, the United States, and as far south as Northern Mexico. The ecology and life cycle of striped skunks, especially in northern latitudes, makes the skunk an effective vehicle for rabies transmission, during the spring breeding period (February-March). Male skunks are polygamous and will attempt to breed with several females. Litter sizes range from five to nine, (1:1 sex ratio usually), with parturition usually occurring from May to June. Juveniles or young of the year disperse from the adult female between July and October. Competition for

communal dens occurs in October and November in colder climates. The winter denning period is variable, but may last throughout December and January. As many as 21 skunks may occupy a single den, with denning sites ranging from ground burrows and culverts, to farm buildings, residential homes and junk piles.

As skunks occupy a variety of habitats from prairie grasslands and deserts to forest edge and urban fields, population densities are highly variable ranging from 0.5-2.5 km² on the prairies to 36/km² in urban areas of Ontario. Skunks tend to be non-aggressive and do not defend a territory. However aggression between males is evident when competing for communal denning sites and breeding females. Home ranges are usually quite small (2-4 km²) especially in cities, however, skunks are capable of dispersing over distances in excess of 100 km.

Population turnover is quite rapid among skunks, juveniles usually account for 60-70% of the summer/autumn population and skunks more than three years of age are a rarity. Skunk populations are limited by diseases such as rabies and leptospirosis, rabies control programmes, trapping and road-kills.

Polygamous males, communal denning, large litter sizes and high pregnancy rates (90%), all part of a successful reproductive strategy, make skunks a species capable of rebounding rapidly from population declines due to diseases such as rabies.

4.4 Physiological markers for baits

Physiological markers are broadly defined as physical or chemical agents which, when orally ingested by animals, will be retained and can be detected for known periods of time after ingestion. These markers are useful for the study of animal movements, feeding behaviour, efficacy of different bait matrices and baiting techniques, and environmental exposure to chemicals. Markers are represented by a wide variety of chemical classes consisting of rather simple inorganics, complex synthetic organics and radioisotopes.

Physical agents include fluorescent pigments (phosphors) such as synthetic special formulations of zinc sulphide, zinc-cadmium sulphide complexes, microscopic coded plastic particles, pigments, and coloured particles which are not fluorescent. In general, advantages of physical agents include low cost, good acceptance by animals, high stability, compatibility with a wide range of matrices, and relative ease of detection. Many fluorescent pigments and coloured particles can be detected in natural light with the unaided eye. Some require microscopic examination under ultraviolet light. A major disadvantage of physical agents is short retention time because they pass through the gastrointestinal tracts of animals in a matter of hours.

Chemical agents that have been or are currently used as markers include tetracycline (an antibiotic), rhodamine B (a potent dye that is a suspected carcinogen), organochlorine pesticides such as mirex, iophenoxic acid (formerly used as a cholecystographic radiopaque medium diagnostic aid), and radioisotopes of manganese, zinc, selenium, iodine, and cesium. A major advantage of chemical agents is that their retention time in animals ranges from several days to several months depending upon the chemical used. A major disadvantage for most chemical agents is that they require sophisticated procedures and instrumentation for detection. Strict licensing requirements and regulations are potential constraints when using radioisotope markers.

4.5 Chemical attractants for caged coyotes

Results of a recent survey of Animal Damage Control (ADC) field personnel showed a strong interest in and need for research and development of chemical coyote attractants. Seasonal responses of captive coyotes (*Canis latrans*) to nine chemical attractants (W-U lure - trimethylammonium decanoate plus sulphides; TMAD - trimethylammonium decanoate; SFE - synthetic fermented egg; FAS - fatty acid scent;

CFA - synthetic monkey pheromone; artificial smoked fish flavour, artificial beef liver flavour, yeast autolysate and decanoic acid) were evaluated. Twenty-six additional attractants were tested only during the summer. W-U lure and FAS produced the greatest total response times from coyotes during all seasons of the year and their overall attractiveness was nearly identical. FAS and smoked fish flavour evoked the most lick-chew-bite and pulling behaviours during the summer and have potential for improving the performance of M-44 devices in warm weather (the M-44 is a tubelike spring-loaded device partially inserted into the ground; the exposed portion is baited with an attractant which, upon being pulled by a coyote, ejects a lethal dose of sodium cyanide into its mouth). A high percentage of all behavioural responses to attractants was spent in rub-rolling activity. For some attractants rub-rolling accounted for over 80% of the total recorded response time. The amount of time coyotes spent rub-rolling appeared to be an index of the attractiveness of a particular attractant. If a control measure could be developed to utilize this response, it might be highly effective.

4.6 Raccoon capture-vaccination-release (CVR) trial and implication for future oral rabies vaccination efforts

In an effort to prevent the North to South spread of terrestrial rabies from the mainland on to the Delmarva Peninsula, the Maryland Department of Health and Mental Hygiene initiated a large-scale trap-vaccinate-release campaign targeting raccoons in 1987. From August 1987 to November 1989, a total of 3 618 individual raccoons were vaccinated against rabies with IMRAB in a 22 500 hectare study site located at the isthmus across the entire northern neck of the Peninsula. The percentage of the raccoon population vaccinated at least once ranged from 43.6% in 1987 to 62.5% in 1988 to 68.5% in 1989. Despite these vaccination levels, rabies breached the vaccination zone in October through December 1989, when infected raccoons were detected 13 km south of the immune barrier. Possible reasons for this include:

- insufficient numbers of individual raccoons captured and vaccinated due to non-target catch (52.5%) and high raccoon recapture rates (48.8%);
- high recruitment of unvaccinated newborns into the population each year (41.2% - 47.9%);
- dispersal of infected juveniles through the vaccination zone; and
- a 2.7% rabies exposure rate in the raccoon population when vaccination first started in 1987.

Implications for success of future oral rabies vaccination campaigns targeting raccoon populations in the mid-Atlantic state region were discussed.

5. MASS PRODUCTION OF BAITS: ADVANTAGES AND PITFALLS

Most campaigns to eliminate rabies from terrestrial wildlife will require the treatment of large areas. That will require hundreds of thousands, or even millions, of baits each year. Therefore, machine-based mass production of baits will probably become essential. This imposes two major constraints on investigators planning research designed to produce a baiting system for wildlife rabies control: (1) the necessity for mass production will limit some of the options in bait design which may be attractive for small-scale trials; (2) since mass production machinery will probably have to be custom-designed, it is important to choose the final bait form carefully because machine design may limit the ability to make changes later.

The Ontario system for mass production of baits requires three machines. The first machine makes blister-packs, fills them with vaccine and seals the lid in place. That is a routine industrial process except for the bio-containment necessary for worker safety. The latter constraint necessitated a custom-designed machine

capable of operating within a biosafety hood but also able to be rolled out easily for cleaning and sterilization. The blister-packs are checked for leaks and loaded, by hand, into metal magazines. The metal magazines are stored overnight at 4°C.

The second machine makes the bait. The basic unit in the machine is an open-bottomed mould which forms three baits. The machine uses 216 such moulds when in full operation. The moulds are initially fitted onto flat plates, which are part of a chain conveyor. This conveyor moves on an indexed system, transporting the moulds by one mould's width on each pass. An automatic pneumatic arm deposits a label in the bottom of each mould.

Further down the line, measured amounts of molten matrix are dropped into the moulds. The temperature of this mixture is very critical: it must be 68°C ($\pm 0.5^\circ$). If the mixture is too warm the blister-pack, which is inserted at the next stage, will float back to the surface when immersed. If the fat is too cool, it will solidify before the blister-pack can be immersed. Blister-packs are deposited onto the surface of the fat mixture by pneumatic arms, then pushed under by separate, heated arms. From here the baits continue on the chain conveyor into a refrigerated tunnel.

As they come out of the tunnel the moulds are near the starting point. At this point, empty moulds are pushed onto the chain and the full moulds are displaced onto a flat track. By this time the baits are solid enough to stay in the mould when it is moved off the bottom plate provided on the conveyor. The full moulds travel around three sides of the machine, through a further cooling tunnel.

Finally, mechanical plates come up from underneath and push the baits clear of the mould. The baits are displaced sideways, and from there they are hand-packed onto cardboard trays. An automatic packer is under development. The cardboard trays hold six rows of 24 baits. These are loaded into specially designed cartons which have slots aligned with slots on the trays to allow rapid circulation of cold air in the freezer. The baits are then frozen, stored and transported at -30°C.

The third machine in the system is mounted in the Twin Otter aircraft for aerial bait distribution. It can drop between 5 and 6 baits per second. The aircraft can carry up to 16 000 baits per flight. Under ideal conditions this allows for treatment of 1 500 km²/day at 20 baits/km². In autumn 1990 the Ontario team is expected to drop 750 000 baits in 3-4 weeks of flying, using two aircraft. All the baits for this campaign were made between April and August 1990, during which time the speed of the bait making machine was increased from 20 000 per week to 42 000 per week.

In Ontario an integrated system has been achieved because of the overriding need to eliminate manual handling of the very large numbers of baits which must be made, stored, transported and dropped. By adding a second storage tank for fat/wax, and by increasing manpower, the above output can again be doubled.

The major constraints imposed by the machine system described above are the following: (i) Any new ingredients added to the bait mixture must be able to flow by gravity and not clog pipes and valves. When experiments were initiated with lower proportions of Microbond (i.e. the wax which imparts strength to the baits but which also might lower bait palatability to wild foxes) it was suspected that the baits might disintegrate as they were pushed out of the moulds. (ii) The other major constraint is cost as the machines described above cost about US\$200 000 to develop, and major changes in design are likely to be expensive.

6. PLANS OF WORK FOR FURTHER RESEARCH

6.1 The Wistar Institute of Anatomy and Biology, Philadelphia, USA

The Institute will concentrate in the coming year on the evaluation of safety of the vaccinia recombinant vaccine (VRG) in the pilot area. This will especially relate to the dynamics of VRG in the field, measurement of bait contacts, and serological response in target and non-target species. The same principles may be expanded to a mainland situation, the State of Pennsylvania being the logical choice for the next trials.

6.2 Laboratoire d'Etudes sur la Rage et Pathologie des Animaux Sauvages, Malzéville, France

The Laboratory will work on increased surveillance following oral vaccination campaigns, and carry out further research on the safety and efficacy of SAG and VRG as well as on bait uptake under field conditions.

6.3 Institute for Arctic Biology, Fairbanks, Alaska, USA

The Institute will test, in collaboration with the rabies unit of CDC, Atlanta, the SAG strain in caged Arctic foxes and major non-target species. Further bait acceptance trials need to be undertaken and the institute would welcome samples of baits from other institutions. The impact on environmental conditions, with special reference to freezing/thawing, will also be evaluated. Placebo baits will be distributed in the summer 1991 near dumping and denning sites to estimate the percentage of foxes which could be vaccinated this way.

6.4 Centers for Disease Control (CDC), Atlanta, USA

The rabies unit at CDC plans to test the canine adenovirus rabies recombinant vaccine for enteric vaccination. A special project aims at acquiring the equipment for measuring iophenoxic acid serum titres so that cheap and rapid results could be obtained. Further studies will be carried out on bait acceptance by dogs in Mexico and mongooses in Puerto Rico. More work is needed in order to better estimate mongoose population size and densities. The fishmeal polymer bait will be tested in mongooses.

6.5 Animal Diseases Research Institute, Nepean, Ontario, Canada

The control campaign will be continued in eastern Ontario. In selected urban areas, a research project combining the Trap-vaccinate-release technique for raccoons and skunks with hand-baiting for foxes will be initiated. Work will be pursued on baits and vaccines (a possible trial with the human adenovirus rabies recombinant vaccine may be initiated). Special attention will be paid to experiments on bait distribution.

In late September 1990 the Ontario rabies team will begin a series of experiments which will be carried out to design the best combination of bait density and flight pattern to achieve maximum acceptance of baits by fox populations. Thirty-one plots, each 15 x 15 km (225 km²) and separated by a minimum of 5 km, have been located in counties east of Lake Huron. The plots were chosen with reference to fox harvest statistics for 1986-1989, to provide the best samples of foxes for evaluation of experiments. The minimum sample per plot is expected to be 40 foxes. Two different bait types, three bait densities and one or two flight lines will be used. Each experiment will be replicated on six plots.

These plots will be used for different experiments in future years, plus some replication of present treatments. The rabies team in Ontario plans to try twice-yearly baiting, starting in spring 1991. There are skunks and raccoons on most

plots so there is an opportunity to try special baits and distribution tactics for those species as well. Collaborative projects in this experimental area are welcomed.

6.6 Denver Wildlife Research Center (DWRC), Colorado, USA

The Center was recently requested to discontinue any work not related to studies for registration of chemical products. However, the Center would like to pursue its work on baits and baiting techniques in the following areas: (a) evaluation of the overall bait uptake obtained with equal numbers of two different baits made available simultaneously at the same locations, (b) to test the central baiting system, and (c) to index target and non-target populations in relation to bait uptake.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Baits and Baiting Strategies

Testing for bait preference of wild animals is a complex subject, with many confounding factors. The participants identified several areas to which special attention must be paid when designing trials.

- a) Bait preference tests should be conducted on both caged and free ranging animals as behaviour may differ within a given species between the groups.
- b) Removal of baits by non-target species should be evaluated.
- c) Researchers are encouraged to choose study areas where other scientists have already accumulated useful information on target and non-target species.

In addition the group stressed the need to conduct further studies to evaluate the feasibility of combining biomarkers with the vaccine chamber. For that purpose placebo trials with biomarkers only in the vaccine chamber should be carried out.

Basic principles for the initiation of bait preference tests in caged and wild ranging animals are listed in the following.

7.1.1 Organisation and implementation preference tests with caged animals

- a) Origin and care of test animals:
 - wherever possible, work should be carried out on local wild target animals rather than on animals from a fur farm. Different species should be maintained separately.
 - appropriate quarantine should be imposed to avoid bringing in rabies and other diseases; acclimation time should be a minimum of 30 days, allowance being made for species differences;
 - care must be provided in accordance with local standard requirements and sound veterinary practices;
- b) Special points to consider when designing experiments:
 - Attention should especially be paid to social hierarchy which may bias the use of a central area for bait testing.
 - Bait preference tests should be done by pair-wise comparisons (i.e., experiments should not involve more than two substances in a single test). Testing for three or more substances in the same trial has been shown to be unproductive, in addition: baits under test should be presented several times for as short a time as practical (e.g., 12-24 hours). This duration may vary according to species.

- The effects of withholding regular food in bait preference tests should be determined;
- no regular food should be available while tests are in progress;
- the nature of the animals' contact with the bait should be recorded according to a preestablished questionnaire. All possibilities should be listed in a multiple-choice type questionnaire. The correct answers could be underlined or circled during the trial. Some examples are given below:
 - (i) level of bait consumption - partial/complete/not eaten
 - (ii) blister-pack
 - rejected/chewed/gulped/swallowed
 - punctured/intact
- c) Interpretation of the results:
 - the size of the group under test should be a minimum of six animals.
 - consultations with statisticians and WHO collaborating centres will help determine how many replications are required;
 - attention should be paid to the effect of the localization of the baits vis à vis animals under test and to the time of day (especially with respect to feeding times) which is an important variable on such trials.

7.1.2 Organisation and implementation of preference tests with free-ranging wild animals

Once trials on caged animals have allowed the selection of a limited number of substances for further experiments, these need to be verified by experiments involving wild, free ranging animals belonging to the target species.

Attention should be paid to: - animal behaviour which may bias particular forms of testing; - interference of non-target species with the baits which may confound results of some forms of experiments. So far experiments have used direct observation, indirect bait disappearance and track stations to evaluate the importance of the factors above.

7.1.3 Designing small and large scale trials:

Important variables should be taken into consideration at this stage. They consist of seasonal and daily variations mainly related to target species ecology. The following questions should be addressed when designing small or large scale trials:

- a) Seasonal
 - When is the best season for immunizing animals?
 - When is the best season for reaching target species with baits?
 - Are logistical considerations important for choosing the appropriate season?
- b) Daily
 - Is there a best time of day to conduct choice experiments?
 - Is there a best time of day to distribute baits?

Means of expressing bait dispersion patterns as well as density must be developed. Attempts should be made to determine the fate of baits carried away from the baiting area.

In addition, attention should be paid to the following when designing small-scale field trials:

- The level of difference to be detected should be defined prior to initiation of trials. A maximum level of 10% (e.g., 60% vs 70%) is suggested. The number of specimens needed to detect this should be predetermined. The size of the area should be chosen next. Attention should be paid to problems arising from emigration and immigration of target animals.
- Indices of abundance of target and important non-target species should be determined before small-scale trials are undertaken.
- The optimum densities of baits needs to be determined.
- For small scale field trials aiming at determining the percentage of the different animal populations that can be reached by baits, a sample of baits placed on tracking stations should be used to reflect the fate of all baits distributed.

7.1.4 Baiting strategies

Carefully designed small scale placebo baiting trials may be useful to determine the feasibility of applying multiple types of baits on a single site in order to maximize uptake in a single target species. There is also a need to investigate whether two or more bait types could be used to optimize acceptance by two or more target species as well as whether two or more attractants directed towards two or more target species can be used in the same bait.

Selection of the optional baiting distribution strategy should be based on the following criteria: the target species' ecological characteristics, experience/success in small-scale placebo trials, most effective use of resources (i.e., money and man power).

Baiting strategies which need to be evaluated include:

- Aerial distribution;
- A combination of aerial and ground distribution;
- Ground "random" distribution;
- Ground in reference to target habitats/signs/trials, etc.
- Central point ("feeding station")

7.2 Intensified rabies surveillance and collaboration in rabies control in the USA

As it is desirable to establish a coherent terrestrial wildlife rabies control programme in the USA, the participants made a specific recommendation towards the establishment of a collaborative wildlife rabies control programme in the USA.

A coherent terrestrial wildlife rabies control programme includes not only the vaccination of appropriate wild animals, but also the necessary surveillance system to ensure its success. The current rabies surveillance in the USA, while appropriate for human and domestic animal health protection, is at present considered inadequate to fulfil the minimal requirements necessary to perform coherent terrestrial wildlife control.

Existing legislative authority, if any, within the Public Health Service and the USDA/APHIS (or other federal agencies), needs to be examined with regard to wildlife rabies control in the USA. If appropriate legislative authority exists, cooperative efforts among agencies should establish the role of each agency in a coherent

terrestrial wildlife rabies control effort. If this appropriate legislative authority does not exist, then either federal mandates need to be established, or at least during an initial phase, state or regional agencies may wish to proceed with available resources.

Depending upon the above outcomes, it is desirable that federal, regional, or state agencies meet to establish the necessary procedures to implement terrestrial wildlife rabies control.

Coordination at the borders, i.e., with Canadian, Mexican and also with agencies of other western hemisphere governments is highly desirable.

7.3 Population ecology and ancillary studies related to oral immunization programmes

7.3.1 Population ecology

The understanding of affected species ecology is an essential component of oral rabies vaccination programmes. The following objectives should be fulfilled prior to oral vaccination (ORV) project implementation:

- Bait densities necessary to reach the target species should be defined.
- A method for evaluating programme efficacy should be determined.
- Cost-efficiency should be maximized.
- Public awareness and understanding regarding programme goals and objectives should be ensured.
- Scientifically valid and responsible information regarding the target population should be incorporated into oral baiting programmes.

Studies on target species ecology conducted prior to an ORV project should include the following steps:

- The presence of the target species in the treatment area should be ascertained.
- The presence of federal or state-listed threatened or endangered vertebrate species in a treatment area should be established.
- Non-target species composition should be determined in treatment areas especially with regard to those species most likely to compete with the target species for bait uptake.
- Existing animal population data applicable to target and non-target species within the area should be used as the basis for ORV project planning.
- In the case of non-existing or inadequate population information, a minimum, relative abundance of target species should be determined in the treatment area.
- It is strongly recommended that state biologists with knowledge of the target species should be integrated during all phases of bait delivery in the treatment area.
- Animal population monitoring was identified as a major component of any ORV project. It is recommended therefore, that rigorous population and ecological surveys be conducted to serve as the scientific basis for validating and verifying monitoring procedures that will be adopted at the regional level.

It is recommended that pre-baiting population sampling occur no more than three months prior to bait delivery and that post-treatment sampling occur within a similar period of time following baiting. The two samplings should be made within the shortest possible time.

7.3.2 Ancillary studies

The following are recognized as main topics for ancillary studies or investigations:

- Serological surveys pre- and post-treatment;
- Establishment of the legal authority regulating the handling, treatment, vaccination or transportation of target species by the public;
- Mortality monitoring and surveillance following treatments.
- The timing of baiting frequency.

7.4 General recommendations

- WHO should invite the scientific community involved in bait delivery programmes to organize and document a protocol for planning and conducting oral vaccination campaigns and include a section on the biology and ecology of target species in North America.
- WHO should consider organizing a workshop on population monitoring and rabies surveillance with relation to oral vaccination projects.
- A centre in charge of the collection of data on attractant and bait matrix substances should be established.
- Standard definitions of commonly used terms should be elaborated.
- WHO should sponsor one or more workshops targeted specifically at particular bait/vaccine/target species situations (e.g., Polymer bait - VRG - Raccoons.)
- In view of the progress recorded in Europe and Canada, WHO should encourage members of each team to visit field study areas or participate in activities of other teams.

ANNEX 1

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ANNEX 2

Table 1. Bait types used in field trials for the oral immunization of foxes against rabies in Europe *

	Chicken head	Tübingen fox bait	Virbac bait	Fish meal polymer	East German bait
Components	chicken head	fat and fish meal	fat and fish meal	polymerized fish meal, wax	fat and fish meal
Size	-	45 · 45 · 13 mm	50 · 40 · 14 mm	55 · 35 · 20 mm	?
Weight	30 - 80 g	≈ 20 - 25 g	≈ 25 - 30 g	≈ 35 - 40 g	?
Vaccine strain	SAD Berne FRG/ITA: SAD B19	SAD B19	SAG 1	VVTGgRAB (Vaccinia rec.)	SAD Potsdam 5/88
Vaccine container	blister made of plastic/aluminum	blister made of plastic/aluminum	blister made of plastic/aluminum	plastic bag	blister made of plastic/aluminum
used in, used from - to, method of bait distribution (m = manual, a = aerial)	SWI, 1978- m (a) FRG, 1983-85, m ITA, 1984-85, m FRA, 1987-88, m	FRG, 1985-, m (a) ITA, 1985-, m AUT, 1986-, m BEL, 1986-, m FRA, 1986-, a (m) LUX, 1986-, m FIN, 1988-, m NET, 1988-, m CZE, 1989-, m	FRA, 1989-, a	BEL, 1989-, m / a FRA, 1990-, a	GDR, 1989-, m

Country abbreviations: AUT = Austria, BEL = Belgium, CZE = Czechoslovakia CSFR, FIN = Finland, FRA = France, FRG = Federal Republic of Germany, GDR = German Democratic Republic, ITA = Italy, LUX = Luxembourg, NET = Netherlands, SWI = Switzerland, YUG = Yugoslavia

References: Rabies Bulletin Europe 13(3), 1989; Kintschner et al. 1990; Stöhr et al. 1990, West European Cooperation on Oral Vaccination of Foxes against Rabies, Luxembourg, January 24, 1990.

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ANNEX 3

Table 2. Bait densities, bait acceptance rate, and percentage of foxes positive for tetracycline *

Bait type	Country	Typical bait densities (km ⁻²)	Bait acceptance rate (average, range)	% of foxes positive for tetracycline	References
Chicken head	SWI	10 - 15 (average: 13.4)	d2 : 51% (26-69%) d4 : 76% (68-84%) d10: 97% (89-100) (n = 863)	average: 60 % (n=7058) range: 45-80 %, depending on accessibility of area and bait density	·Antognoli 1988 ·unpublished data of the Swiss Rabies Rabies Center
	FRG	15		average: 75 % (n=1680) range: 68-90 %	·Schneider & Cox 1988 ·Schneider et al 1988
	ITA	10.7	d4 : 25.5 - 31.8 % d8 : 41.8 - 68.1 % d14: 61.1 - 85.2 %	63 % (n=46), after campaign in autumn; 43 % (n=21, spring)	·Balbo & Rossi 1988
Tübingen fox bait	FRG	15 20 in Nordrhein-Westfalen	d14: 42% (31-54%) d14: ? (65-85%)	average: 73 % (n=933) range: 65-81 %	·Schneider & Cox 1988 ·Schneider et al 1988 ·Zwingmann, pers.com.
	ITA	11.0 - 13.0	d4 : 17.9 - 20.6 % d8 : 34.7 - 41.1 % d14: 50.8 - 60.2 %	35.5 % (n=107) and 42.2 % (n=199); both samples from summer	·Balbo & Rossi 1988
	BEL	5 - 15 average: 11	d14: 57 - 72 %	47 - 69 % (n=124)	·Brochier et al 1988
	LUX	15	d4 : 24% (21-31%) d8 : 46% (43-52%) d14: 63% (58-66%)	62 - 71 % (n=271)	·Frisch et al 1988
	FIN	15		91 % in foxes (n=67) 75 % in raccoon dogs (n=156)	·Westerling 1989
East German bait (placebo)	GDR	15 - 20 (?)	d3 : 35 % d6 : 73 % d9 : 86 % d15: 92 %	> 70 % (n=?)	·Stöhr et al 1990

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