ORAL RABIES VACCINATION IN THE AMERICAS

ATLANTA - November 5-6, 1990

November 5

8:00 - Inauguration

8:05 - Overview - Why do we need population estimates for Oral Rabies Vaccination? - Nettles

<u>Animal Population Estimates</u> Rapporteur - Linhart

Foxes

8:15 - in Ontario - Nunan

8:30 - in Alaska - Follmann

Raccoons

8:45 - on Parramore Island, Va. - Hanlon

9:00 - on Sapelo Island, Ga. - Hon

9:15 - in Maryland - Garner

9:30 - in Pennsylvania - Hayden

Skunks

9:45 - in the city of Toronto - Rosatte

10:00 - Discussion of Population Estimates in Foxes, Raccoons, and Skunks.

10:30 - **Break**

Mongooses

11:00 - in the Dominican Republic - Lord

Dogs

11:15 - in Brazil - Belotto

11:30 - in Atlixco, Mexico - Fishbein

11:45 - Roundtable Discussion of Population Estimates in Mongooses and Dogs

12:15 - **Lunch**

<u>Baits, Bait Distribution and Markers</u> Rapporteur - Johnston

Red Foxes

1:45 - Ontario - Bachmann

Raccoons

2:00 - Parramore Island, Va. - Hanlon

2:15 - Pennsylvania - Buchanan

2:30 - Sapelo Island, Ga. - Linhart

2:45 - Mass Production of Polymer baits - Smith

Skunks

3:00 - Canada - Wandeler

3:15 - **Break**

3:45 - Roundtable Discussion of Baits, Markers, and Bait Distribution in Foxes, Raccoons, and Skunks.

Mongooses

4:15 - Puerto Rico - Vargas

Dogs

4:30 - Atlixco, Mexico - Fishbein

4:45 - Atlixco, Mexico - Linhart

5:00 - Roundtable Discussion of Baits, Markers and Bait Distribution in Mongooses and Dogs

November 6

Oral Rabies Vaccines in Development: Progress Reports -

Efficacy, Safety, Stability, Transmissability

Rapporteur - Wandeler

8:00 - Vaccinia-RG - Rupprecht

8:15 - Raccoon Poxvirus-RG - Esposito

8:30 - SAG - Follmann/Fishbein

8:45 - Human Adenovirus - Wandeler

Surveillance of Rabies and its Use in Disease Control

Rapporteur - Tinline

Foxes

9:00 - Ontario - Tinline

9:15 - Alaska - Ritter

9:30 - New York - Trimarchi

Raccoons

9:45 - Virginia - Jenkins

10:00 - Pennsylvania - Rupprecht

10:15 - **Break**

Skunks

10:45 - Ontario - Casey

11:00 - Roundtable Discussion of Surveillance of Rabies in Foxes, Raccoons, and Skunks, and its Use in Disease Control

12:00 - Lunch

Mongooses

1:30 - Dominican Republic - Vargas

Dogs

1:45 - Brazil - Schneider

2:00 - Mexico - Garza

2:15 - Roundtable Discussion of Rabies Surveillance in Dogs and Mongooses, and its use in Disease Control

2:45 - **Break**

3:15 - General Discussion of Coordination between the various groups

FOXES - Canada, Alaska

SKUNKS - Canada, U.S.

RACCOONS - Virginia, Pennsylvania, Georgia, Canada

MONGOOSES - Puerto Rico, Dominican Republic, Grenada

DOGS - Mexico, Brazil

6:00 - Adjournment

ORAL RABIES VACCINATION IN THE AMERICAS

Atlanta, Georgia   November 5-6, 1990

The meeting was opened by George Baer who welcomed the participants to the first meeting on 'Oral rabies vaccination in the Americas'.

He began by reviewing the efforts of Frans Steck and Alex Wandeler, who in 1978 initiated the landmark oral rabies vaccination of foxes in Switzerland, an effort that has resulted in the elimination of rabies in that country, with similar programs now under way in many other European countries and Canada. But in planning field programs to vaccinate other wild animals and dogs it will be crucial to take into account the vast differences in population numbers, baits, and bait distribution, besides, of course, vaccine efficacy and safety. The early and continued success of the Swiss program led to its continued support, but unless early efforts are made to immunize enough animals of other species to stop transmission - and this means the needed number of baits distributed at the lowest price possible - it will be more and more difficult to get the political and financial support to continue the programs. In the two day meeting rabies researchers from Canada, Brazil, Mexico, the Dominican Republic, and the U.S., are to describe work with five species of animals: red foxes, arctic foxes, skunks, mongooses, and dogs. Hopefully these discussions will lead to a greater understanding of the problems we are facing and the ways to overcome them. It is also hoped that this will be the first of a series of annual meetings in different parts of the hemisphere.

This meeting would not have been possible without the ample assistance of the Rockefeller Foundation, which supported many of the international participants to the meeting.

Vic Nettles began the meeting by stressing the importance of knowing population densities for oral rabies vaccination for the following reasons:

- To relate bait densities to the target species
- To relate bait densities to non-target species
- To know something about population densities as related to the initiation of the program (questions from the press, for instance).
- To relate what occurs after the baiting to the possible effects of the vaccine on the target species
- To develop models for similar trials

The program continued with Sam Linhart as rapporteur of the session on ANIMAL POPULATION ESTIMATES.

The first presentation was by Chris Nunan (Canada) who discussed population densities of red foxes in Ontario. There are various ways to calculate population densities, including counting actual numbers (virtually impossible), mark-recapture techniques to obtain a Lincoln Index, and other types of indices, each of which require certain assumptions to reach a valid population estimate, both for closed or open populations. Some of the indices used in Ontario have been harvest surveys and transect/quadrant sampling questionnaires distributed to farmers in the area; the replies to these questions allow one to draw indirect population estimates. It is important to raise only those questions that need answering, and to demand the proper accuracy in the replies given (the techniques are reviewed in Krebs book on Ecological Methodology). The experience with the use of such estimates in Ontario have permitted workers to develop a model for use there; the estimate of population in areas with good habitat is 1 fox/$km.^2$.

The next presentation was by Erich Follmann who discussed his work with arctic foxes, including the distribution of rabies in Alaska. There is no good estimate of fox numbers because of the vastness of Alaska, and because of variations in reporting. Another problem is ice formation which covers many areas where foxes feed on seal carcasses. In northern Alaska the territories of foxes are only seasonally maintained, and this complicates any 'census' which is attempted. Rabies tends to occur around tundra areas where foxes den, and those dens can be relatively easily located because of the high amounts of nitrogen from fox urine and feces, resulting in greening of plants. Moreover, many of the same dens are used each year and thus can be easily identified. The distribution of baits would most likely be in preferred areas, where rabies cases have occurred and where dens can be easily identified.

Cathy Hanlon continued with the first of three presentations on raccoon population estimates. Rabies is a density-dependent disease, and one needs to know the number of baits needed in relation to the population density. There are many raccoons on Parramore Island, an island off the coast of Virginia. Before the studies described there were no data on the populations of raccoons there. In order to estimate the population there a mark/recapture method was used as a basis for deciding whether to bait the island. It was desirable, in this case, for individual animals to get multiple baits. A modified Peterson Lincoln index was used. The estimate, based on the data collected, was that there was 1 raccoon/2.7 hectares. Estimates of the Parramore Island raccoon population were obtained in 1987 and 1990.

Tip Hon continued with a discussion of raccoon population estimates on Sapelo Island, Georgia. He described the physical characteristics of the island and discussed prior knowledge of raccoon populations there. The best way to get a quick and rough estimate of raccoon and fox population trends is through their rate of visitation to scent posts, each 'treated' with fox and bobcat urine, and placed every 2/10 of a mile apart; non-target species such as opossums also frequently visit stations. The mean rates for grey foxes was 15% (of scent posts visited) to give an index of 150; raccoons, on the other hand, had a mean of 7.5% to 8.0%, giving an index of 75-80. When one reviews the index levels by the whole state vs. Sapelo Island a ratio of approximately 30% is found for both. Hon ended by describing how he calculated the number of baits distributed on Sapelo Island were calculated.

Arnie Hayden described the large harvest of raccoons in Pennsylvania (in the 1920s the raw fur value was $20.00). Until the 1930s raccoons were not so abundant. In the 1940s there was a habitat change (mature forests), with animals very abundant until the 1990s; 800,000 were harvested ($19/mi^2$) in the 1970s. The sale price affects the harvest. At present the harvest is 150,000 annually because the price is low. State game lands (SGLs) were selected to study raccoons in relation to habitat and harvest patterns; they averaged 1000 acres. Animals were live-trapped, aged, sexed, radioed, bled, and their incisors pulled. By trapping 40-50 days until few new raccoons were captured the populations could be estimated. The population was $41.4/km^2$ in agricultural areas while in forested areas it was only $11.9/km^2$. A population which has been reduced will fully recover in two years. The density of trees and other habitat factors relate to raccoon densities, and the diversity of habitat is a key factor that affects raccoon populations; mature forest stands in bottom lands were the most preferred habitat. In mountainous habitat there is little movement between drainages.

Rick Rosatte began the session on skunk populations. He stated that there is a critical density at which rabies transmission occurs, he mentioned five reasons why skunk densities are useful in rabies control, reviewed various ways to estimate densities, and different ways to look at the data (Peterson, Jolley, etc.). Various assumptions have to be made to obtain population estimates, and it is important to use the same criteria for comparative studies (the period of trapping, bait type, trap nights, length of trapping period, etc.). From 1984 on they have studied urban skunks and raccoons in Toronto using a trap-vaccinate-release program which has also included the collection of blood, incisor teeth, and information on the sex and age of the animals trapped. He discussed the annual activity cycle of skunks in relation to the density estimates obtained by line-capture-mark-release. The home ranges (small) affect the density estimates; that is, if home range and movements are large the animals may not be present when the area is being trapped, resulting in an underestimate of density. The mean home range for skunks and raccoons in Toronto is around .5 km^2. Mean skunk densities in Toronto were between 3-6 /km^2. Raccoon densities were about twice that of skunks. Densities were habitat dependent, that is, most skunks were found in fields in Toronto, while most raccoons were located in forest-park and residential areas. The annual mortality of skunks and raccoons in Toronto is 40-60% (many are killed by vehicles in the fall). He discussed rabies cases in relation to vaccination efforts. Since 1987 only 3 cases of rabies in skunks were reported in the trap-vaccinate study area, whereas 45 cases were reported the previous 6 year period. In preparing for future oral vaccination efforts skunk baits were distributed at 25, 50, 75, and 99 baits/km^2 in attempts to reach a significant percentage of the population. Acceptance was low (<35%). In small trials on 1 km^2 plots, 147 baits/km^2 were needed to reach 68% of raccoons in a forested park area of Toronto. Raccoon density in the area exceeded 50/km^2, which accounted for the high number of baits needed. A discussion of population estimates in foxes, raccoons and skunks ensued.

Rex Lord presented his experience on mongoose populations in the Dominican Republic. A knowledge of population dynamics is important to determine the actual number of mongooses present. We eventually will need to know how frequently oral vaccination programs will be needed before homing in on mongoose population dynamics and estimates. Mongoose rabies tends to be 'migratory', an important factor in rabies control. The mean number born per birth is 4.4/female/year. The annual population turnover is 57%. Lord used the lens technique to age mongooses because tooth layers cannot be depended upon for reliable estimates. The mean life span of males is 22 months and of females 20 months. One half the population is less than a year old. There is an uneven sex ratio because males have a higher mortality rate. Population densities vary from one animal/ hectare (ha) to 10.4/ha, the mean being 5/ha. The mean home range is 3.7 ha., but it is larger for males. If baits are to be applied the number of mongooses estimated per square km. is 500; it is still not known how many baits would be needed for that population. He showed slides of various habitats in relation to rabies outbreaks; the humid subtropical habitat is where the greatest number of rabies cases occur, with a seasonal occurrence. Tracking boards are used to establish frequency of visitation and population estimates.

The session on canine populations was begun by Albino Belotto who discussed dog vaccination and population estimates in Brazil. The biggest problem is that there are no good techniques for estimating canine populations. One possible way would be to modify the techniques used for estimating human populations (census, etc.). Truly feral dogs are uncommon (as opposed to community dogs). The problem is to measure the community dogs. It is not practical to make a precise census of all dogs. Up to now the number of doses of vaccine needed for programs was based on the estimate of one dog for every ten people (data from other countries). Another way to estimate the number of doses needed was to obtain data from previous dog vaccination programs, allowing, then, for resources and vaccine to be more accurately used. Charts of the human/dog ratio were then developed, and the global ratio of 1:10 was 'pretty close'. Regional numbers, however, varied widely, even within cities. In rural areas the mean number of persons per dog was 6.3 (range 3.7 to 17.7). In Belo Horizonte the mean age of dogs was 2.5 years.

Dan Fishbein discussed the study on dogs and rabies vaccination strategies he was involved with in Atlixco, Mexico, in collaboration with personnel from the local, state and federal health agencies. A door-to-door census was made and a Lincoln index was derived from the ratio of dogs with collars (vaccinated with rabies vaccine during the campaign) and those without collars. Seventy-six percent of the dogs had collars, the same percentage as those vaccinated.

BAITS, BAIT DISTRIBUTION AND MARKERS

Peter Bachmann opened the session with a discussion of the baits used for foxes in Ontario. Baits developed to deliver ERA vaccine (the type used in Canada) include wax-coated sponges and wax-tallow blister-pack cube baits with tetracycline as the principle biomarker used. Bait distribution in the field was done in the past by Cessna aircraft, with 1500 - 3000 baits hand-dropped per flight, or by Twin otter aircraft, with 15,000 baits dropped per flight by baiting machine. Current baiting programs cover 38,000 km^2, one third of the endemic area in Ontario. Bait lines are 1 km. apart. The bait density is 20 baits/$km.^2$. With current mass production machines 20,000 blister-pack baits can be produced per day. One Twin otter airplane can bait up to 2,000 km^2 /day. In 1989, the first year of large-scale baiting in Ontario, approximately 50% (N=343) of the foxes collected from baited areas showed evidence of tetracycline deposits, an indication of bait uptake. In previous small-scale aerial experiments 60-65% of foxes accepted baits. About 17% (N=190) of foxes from non-baited areas had dental tetracycline, indicating egress from and perhaps ingress to the baited areas. Both conditions could lower the actual bait acceptance calculations. In 1989 approximately 40% of tetracycline positive foxes developed immunity to rabies and 10% of foxes without tetracycline deposits showed immunity. This suggests that some foxes are getting enough tetracycline from the bait to mark the teeth, but not enough vaccine to immunize, and in other cases a suitable amount of vaccine but no tetracycline. The issue is confounding and results are not always clear. The natural background antibody levels range from 1-3% in Ontario. The annual cost of rabies to Ontario is $25 million.

Ray Buchanan discussed raccoon baiting on Parramore Island, Virginia, the site of the first U.S. field trial with recombinant vaccinia - rabies glycoprotein vaccine (VRG). This Atlantic coastal barrier island was chosen for its isolation, high security, high raccoon population, low non-target population, and absence of rabies. The bait selected for the trial was a fishmeal polymer cylinder surrounding a one ml. wax ampule containing the VRG vaccine. The polymer cylinder was placed in a polyethylene bag printed with a warning label. A slurry (30 ml.) of blue crab, eggs and water was added to each bag to complete the bait package. A total of 3,120 baits were hand-placed and 'flagged' at 25 m. intervals over 6 test areas on August 20, 1990.

Cathy Hanlon continued the discussion of the work done on Parramore Island. The biomarkers originally tested for use in the raccoon baits were physical markers such as rhodamine B and systemic markers such as tetracycline and fluorescein yellow, and serum markers such as sulfamethazine, sulfadimethoxine, and iophenoxic acid. Rhodamine B quickly washed off the raccoon fur; fluorescein yellow gave variable results; tetracycline, although permanently located in calcified tissues, required tooth extraction or euthanasia for detection. The laboratory analysis for detecting iophenoxic acid was very expensive and even dangerous. Sulfadimethoxine was easily and quickly tested in serum samples by an inexpensive card test but the levels only persisted for a maximum of 7 days. Tetracycline and sulfadimethoxine were selected as the best available markers for use in the field baits. Of the 12 raccoons live-trapped 6 days after baits were placed 11 (92%) were tetracycline positive and 42/53 were positive for sulfadimethoxine (79%), indicating a very high level of bait acceptance. Of the non-target species examined for tetracycline (2 red fox, 3 deer, 9 house mice, 7 rice rats and 4 meadow voles) only 1/9 mice was tetracycline positive.

Sam Linhart described the baiting trials he carried out on Sapelo Island, a coastal island of 10, 572 acres on the coast of Georgia with a high raccoon population and a low non-target population. A polyurethane sleeve bait was selected for the field trials; the bait was made up of a polyurethane foam cylinder surrounding a 2 ml. paraffin wax ampule. The sleeve was precoated with corn muffin batter mix and deep-fried in corn oil. Iophenoxic acid (10 mg./ml.) was placed in the ampules as a serum marker. Preliminary laboratory trials had indicated that this was a suitable bait for delivery of raccoon poxvirus-rabies glycoprotein vaccine to raccoons. Direct field observations indicated that 84% of field baits were taken within 2 nights of placement, 44% by raccoons and 36% by non-target species, indicating that this bait was acceptable to wild raccoons in the field.

Alex Wandeler made the only presentation on skunk baits. At present there is no oral vaccine and bait combination that will immunize skunks efficiently. The vaccines tried have included ERA, SAD, and VRG. A recombinant human adenovirus 5 - rabies glycoprotein recombinant (from ERA) has shown promise in preliminary trials. Seven of 8 skunks developed neutralizing antibodies after the vaccine was dropped directly in the mouth, 2/8 after feeding the 'Canadian blister-pack', 5/8 by wax-coated sponge bait, and 1/7 with the fishmeal polymer bait. A small field trial with blister-pack baits with codfish oil attractant indicated good acceptance (34/50 skunks, or 68%). This bait was also attractive to raccoons. Chlortetracycline marker was successfully incorporated into blister-pack baits to simulate vaccine, rather than into wax-tallow baits to simulate vaccine in the blister-pack.

A discussion of baits ensued: Dan Fishbein asked about the number of baits/km^2 required to vaccinate a sufficient number of raccoons to reduce rabies; Chuck Rupprecht replied that the Parramore trial was to test vaccine safety and not its efficacy; Dave Johnston said that there had been various bait distribution trials in Washington, D.C., Maryland, Ontario, Pennsylvania, and Virginia, with uptake rates as high as 60-70%, but more studies are needed to determine the optimum bait density for different population densities and habitats. This is a crucial point: no baiting program will be successful if so many baits are needed that they will make the program prohibitively expensive. Chuck Rupprecht asked about the number of contacts humans had had with baits in Canada; Peter Bachmann replied that in the distribution of over 750,000 baits there were less than 20 contacts, and none were severe enough to require any treatment. Albino Belotto asked about the total cost of rabies in Canada, and was told by Bachmann that Ontario has 80% of the rabies cases in Canada, at a cost of about $25 million annually, including human and animal vaccination. The estimated cost of the oral vaccination program in Ontario is $2.4 million/annum over a 5 year research phase (1989-1994), in which the principal concerns were baiting and baiting strategies, other control methods, vector biology and new oral formulations, especially for skunks and raccoons. The present cost of vaccine-bait manufacture and distribution is $0.83 (Canadian) per bait. This may still be inflated due to developmental costs. Regarding markers, Kathy Hanlon mentioned that tetracycline doesn't always mark raccoons, and removal of the first premolar, for instance, indicated marking rates as low as 20%, and in the canine teeth as low as 54%. Dave Johnston added that the first premolar is not the tooth of choice, especially in older animals; tetracycline marking depends on the animals' metabolic rate, with slower growth in the winter resulting in a lower deposition rate, and with older animals not marking as well as younger ones. Ideally, tooth cementum, dentine, and bone should all be examined for optimal results. Arnie Hayden added that "In some of the studies we have heard, the low bait uptake by females in the spring may be due to the decreased home range and movements of the females when they have young". Rupprecht ended the discussion by asking to clarify a point about VRG in skunks: The original trial with Charlton used VRG grown on BHK_{21} cells and gave good results, while later trials with

VRG grown on VERO cells worked less consistently, and the question of its efficacy in skunks was still not determined properly.

Camilo Vargas presented his work on mongoose baits. In developing a bait in Puerto Rico 11 baits were tried, including yucca mandioc, egg-flavored sponge, Canadian blister-pack, fishmeal polymer, and fish-flavored sponge. The last bait was the preferred one. Potential vaccine containers tested included polyvinyl capsules, a polyethylene pouch, a sponge container, and a wax ampule; the last proved best. In order to get a census of mongoose populations scent stations were baited with various attractants including trimethyl ammonium decanoate and an organic fish compound which proved to be the best attractant. Vinyl floor tiles with a 1:1 mixture of printing ink and oil were used to record animal tracks. Population estimates varied widely, with some reaching $500/km^2$. Dimethyl phthalate was found to be an excellent insect repellent for baits without affecting mongoose acceptance. About 18% of wild mongooses had rabies antibody, but 67% of those over 24 months of age had antibody.

Estrella Flores Collins presented the results of studies in Puebla, Mexico. Three towns of approximately 1,500 people were selected to study the dog:human ratio which varied from 1:2.6 to 1:4; the dog vaccination rate in these three towns varied from 71% to 86%. In baiting trails carried out in one of those villages 5 bait types were used, and the acceptance rate was: Canadian wax-tallow blister-pack bait 10%, fishmeal polymer bait 50%, Denver corn fried batter bait 67%, Denver fish flavored sponge bait 69%, and commercial dog bait 88%. Long corn baits were better than short ones, and dogs tended to gulp soft baits. Dogs were wary of ampules in baits and sometimes left ampules, but it was estimated that 87% of the dogs that chewed baits would have been vaccinated. With further refinement of the baiting system it should be possible to vaccinate more than 70% of the dog population in the towns studied.

ORAL RABIES VACCINES IN DEVELOPMENT: PROGRESS REPORTS - EFFICACY, SAFETY, STABILITY,AND TRANSMISSIBILITY

Chuck Rupprecht reported on the first American field trial with a vaccinia rabies glycoprotein (V-RG) recombinant. V-RG was studied during the past seven years in laboratory trials in the Wistar Institute. It is efficacious against street rabies virus infection in a variety of major vector species and its innocuity has been established for over 30 different non-target species in the laboratory. The field trial on Parramore Island, Virginia, was designed to obtain further information on safety. On August 20, 1990, a total of 3,120 vaccine-laden fishmeal polymer baits were distributed over a 312 hectare section of the island. The bait acceptance rate was over 90% by 96 hours, and 79% of the raccoons captured in the first 6 days had levels of sulfadimethoxine, a serum biomarker. No morbidity nor mortality was observed. No V-RG virus was isolated from the raccoons (by oral or fecal swabs) nor from non-target species. Further analysis is in progress.

Joe Esposito presented information on a raccoon poxvirus rabies glycoprotein recombinant (RCPRG). Raccoon poxvirus was originally isolated from two apparently healthy raccoons. It is quite different from other members of the poxviridae family. Of 600 raccoon serum samples from the DelMarVa area in the late 1980s 8% had neutralizing antibodies against poxviruses. RCPRG induces a good rabies immune response in raccoons, foxes, bobcats, dogs and cotton rats. Skunks and mongooses respond poorly. Tests in mice and rhesus monkeys suggest that RCPRG is less pathogenic than a Copenhagen vaccinia-rabies recombinant (made at the CDC).

Dan Fishbein reported on experiments with SAG (SAD avirulent Gif) in laboratory mice. SAG is an escape mutant selected with monoclonal antibodies from SAD. It is no longer lethal to weanling mice after intracerebral inoculation but is lethal to suckling mice by the intracerebral route (titer 10^9/ml.) and orally (titer $10^{5.5}$/ml.) Vaccinated adult mice are protected when challenged intracerebrally with CVS.

Erich Follmann fed SAG to 6 arctic foxes, and reported that the virus could be recovered from oral swabs only one hour after feeding. Four of the 6 vaccinated foxes showed a good serological response while two had lower responses, but all resisted a challenge with an arctic strain of street rabies virus.

Alex Wandeler and Ken Charlton presented data on a human adenovirus 5 rabies glycoprotein recombinant (Ad5-RG1). This is the only vaccine that immunizes skunks after oral vaccination. Vaccine virus is excreted in the feces for a few days after feeding. There is still no good bait for skunks. Only half the skunks showed seroconversions when the vaccine was given directly into the small intestine with an endoscope. The vaccine did not cause clinical signs nor pathological lesions when given orally to a number of species, but it caused interstitial pneumonia when given intranasally or deposited into the trachea of mice or skunks.

SURVEILLANCE OF RABIES AND ITS USE IN DISEASE CONTROL

Ed Harvey presented Roly Tinline's paper on foxes in Ontario. Testing of foxes for rabies is done to help make decisions on human treatment. It is known that the real number of fox cases is underreported, however; through computer modelling it is estimated that only 10 to 20 percent of actual cases are reported. There is also a built-in delay in obtaining data. The advantages of the Ontario system include its consistency because investigations and reporting are done by District Veterinarians. Also, the use of a point code system gives a more realistic picture of outbreak areas, allows for analyses of spatial and temporal relationships, and permits a correlation to be made between incidence and habitat.

Don Ritter presented the history of fox rabies in Alaska, and pointed out the 3 to 4 year cyclic nature of the disease, as well as the geographic concentration along the coast with sporadic cases transported to the interior. Most human post-exposure treatments result from exposure to dogs. The institution of a lay vaccinator program for dogs has reduced the incidence of human post-exposure treatment there.

Chuck Trimarchi reported that the following indices were indicative of fox rabies outbreaks in New York: foxes with porcupine quills imbedded in the muzzle, increased rabies in domestic animals, and increased exposure of humans. He felt that

- trapping was not a very cost-effective way to obtain positive specimens
- that testing roadkills lacked sensitivity
- that animals that acted strangely were more likely to be positive
- that the most sensitive choice was to test foxes that exposed a person or domestic animal.

The latter, however, meant an important segment of the rabid fox population would be overlooked. Numbers drawn from surveillance are not valid for making conclusions due to the biases associated with the selection of specimens. In New York skunk rabies tends to be associated with red fox rabies and disappears when fox rabies disappears.

Suzanne Jenkins reviewed the advantages and disadvantages of rabies surveillance systems that are carried out to make public health decisions. She then discussed the results of raccoon rabies studies in Virginia. The most recent data were from approximately 3000 raccoons submitted between 1984 and 1988; positive raccoons constituted 34 percent of those tested; the peak months for rabid raccoons were in February and March while the lowest numbers were in May, June or July. A raccoon was more likely to be rabid if it did not expose a human, did expose another animal, was an adult, especially in June through November (juveniles were more likely rabid in December through May), was killed or found dead (not a road kill), or was aggressive, especially in June through November. Serosurveys have not been revealing in Virginia, but the examination of monoclonal antibody patterns in isolates from the field has helped identify sources and define separate skunk and raccoon outbreaks.

Chuck Rupprecht reviewed the results of rabies surveillance in Pennsylvania. He emphasized the need to increase and improve surveillance to help make decisions for oral vaccination programs. Some of the problems associated with reliance on the usual rabies testing policies include changes in submissions when communities "learn to live with rabies", inaccurate testing when laboratories are overloaded, or missing a rabies outbreak because trapped and roadkill animals are not tested. He gave examples of the Wistar Institute's active surveillance in detecting rabies cases that would have been missed, and of the value of serosurveys for rabies in Pennsylvania.

Al Casey reviewed skunk rabies surveillance in Canada, where peaks of rabies cases occur in March, April, and December. Skunk rabies occurs as an extension from the midwest United States and in association with fox rabies endemic/epidemic areas.

The discussion centered on the surveillance done for public health reasons versus the kind of surveillance required for oral vaccination programs, especially after a vaccine is in the field.

Mal Smith from Dupont discussed the polymer baits that were used in the Parramore Island field trial.

Camilo Vargas reported on rabies in Puerto Rico. The most commonly reported rabid animals are mongooses, then dogs, cattle and cats. Most human post-exposure treatments are due to dog exposures, followed by mongoose exposures. There is a great deal of underreporting and a need to organize the data well.

Maria Cristina Schneider pointed out that in Brazil the human rabies trends follow those of dogs. Approximately 40% of the people who are bitten get post-exposure prophylaxis. Brazil has intensified the rabies control program by initiating one day national rabies vaccination campaigns supported by a variety of government organizations, including the military and 'volunteer' organizations. Approximately eight million dogs and one million cats are vaccinated annually. After the vaccination program began the number of rabid dogs and human cases declined markedly and the positivity rate for dogs went from 40% to 11%. A program algorithm has been developed using the following classification for rabies areas: productive - silent - vulnerable - receptive.

Juan Garza reviewed the rabies statistics for Mexico. In 1989, 65 human cases were reported. The average for the previous five years was 71 per year. Seventeen states in central Mexico reported over 70 percent of the rabies cases. Sixty percent of the cases occurred in towns of less than 10,000 inhabitants, 82 percent are from dog exposures, 7.5 from bats and 1.6 from cats. Human rabies deaths almost always occur in people who received no treatment. The quality of the laboratory diagnoses are questionable in half the laboratories. Approximately 35% of the samples tested are positive. Of the 111,000 animal bites reported, 44,000 resulted in post-exposure treatment. In 1989, 6.9 million doses of rabies vaccine were administered to dogs. Animal control is needed but made very difficult because of the distribution of rabies cases in many small towns.

There was discussion of the risk of using oral rabies vaccines in dogs that had close contact with people.

RECOMMENDATIONS, BY INDIVIDUAL ANIMAL SPECIES

FOXES - Child, Fielding, Follmann, Harvey, Johnston, Nelson, Orciari, Ritter, Smith

A. Fox Population Estimation and Census Methods

1. Direct and indirect evidence of population status should be used to predict population levels, including scent stations, track counts, hunting and trapping statistics, and questionnaires.

2. In order to predict potential outbreaks the evidence of fox abundance from game information (by agricultural

and natural resources agencies) should be coordinated with rabies incidence data from public health agencies.

3. Outbreak predictions should be used to plan control strategies for the designation of resources such as human and domestic animal vaccines and oral vaccines for wildlife.

4. For long term planning historical rabies records should be used to predict local rabies cycles .

B. Fox Oral Rabies Vaccine Baiting Systems

1. Because the actual bait that carries the vaccine is only one component of the entire rabies control system, design of the whole process should be integrated from the beginning of vaccine production through to final field distribution.

2. The temperature and climate where the bait is to be stored should be carefully evaluated. Materials produced at room temperature in the laboratory may not perform as well in extremes of heat, humidity, rain, or cold.

3. The natural foods of the target species should be known for the habitat to be treated so that bait attractants can be palatable for target species and as unpalatable as possible for non-target species, including insects and humans.

4. The matrix of an oral vaccine bait should be as homogeneous as possible to prevent loss of the vaccine carrier such as the ampule. Inclusions of various sizes will be detected and rejected at different rates by different species. Red foxes reject hard inclusions above 5 mm.in diameter, whereas dogs may accept larger inclusions.

5. The vaccine and any biomarkers used should be mixed as homogeneously as possible to avoid false correlations.

6. There is still a need to find biomarkers that are compatible with vaccines, detectable via biopsy, easy to process, and inexpensive.

7. The cost of an oral vaccination program can be reduced by carrying out preliminary field trials using baits with only biomarkers to determine target species acceptance rates and distribution strategies. This allows verification of bait effectiveness in a new treatment location.

C. Fox Rabies Surveillance

1. All rabid wildlife should be identified by species. This is especially important where species ranges overlap, such as those of the arctic and red fox in Alaska and Northern Canada.

2. Rabies cases should be as precisely located as possible by use of UTM or military grid coordinates. Location by postal address or town can give misleading location of rabies foci.

3. Fox rabies distribution should be examined for correlations with the distribution of different habitat types or other mammalian species. Many geographical anomalies exist in the distribution of rabies in North America which may provide clues to wildlife transmission mechanisms.

4. The study of the monoclonal antibody profile of rabies strains from wildlife species should continue. These studies can elucidate the transmission of virus in wildlife, such as red foxes infected with arctic rabies strains, and grey foxes infected with bat viruses in New York State.

5. Rabies epidemiology in arctic, grey, and red foxes should be studied using the deterministic and stochastic computer models now available.

<u>SKUNKS</u> - Wandeler, Charlton, Rosatte, Casey, Niezgoda

A. Population Estimates

1. All efforts should be made to obtain data by specific skunk species.

2. For population estimates one should use capture/recapture or capture/observation techniques; a different technique should be used for 'back-up' such as night lighting of marked vs. unmarked animals, or other suitable ones.

3. All these efforts should involve the participation of persons from various areas of the country so that more complete data can be collected and advice provided.

B. Baits

1. Continued study is necessary to obtain a type of bait and attractant needed to have the most effective bait acceptance and uptake. These should include biomarkers

which are effective, long-lasting, and easily read in teeth or bones; their inclusion is necessary to assure bait uptake.

2. The bait and the formulation of the container should be designed to assure effective delivery of both biomarker and vaccine.

3. Consumption by non-target species should be held to a minimum.

4. The efficacy of the vaccine needs to be adequately field-tested.

C. Surveillance

1. All suspicious skunks should be submitted for rabies diagnosis whether they have exposed humans or not; that should be emphasized to police, health authorities, veterinarians, humane societies, and other groups.

2. If surveys are performed separately the results from those surveys should be reported under the 'official' system.

RACCOONS - Nunan, Rupprecht, Buchanan, Jenkins, Linhart, Miller, Smith, Bachmann

A. Population estimates.

1. Simple methods such as scent post and road kill counts should be tested to estimate populations rather than complex methods.

2. All attempts should be made to correlate raccoon density with the habitat in a given area.

3. A basic census method is needed for non-target species.

4. A 'raccoon' model should be developed, similar to the 'Ontario' model for foxes; a group of experts should be immediately gotten together in a working session to arrange this.

B. Baits

1. Tests should be carried out to select the most appropriate vaccine container.

2. A species-specific bait is recommended rather than one

suitable for many species.

3. A variety of scents should be tested with the fishmeal polymer bait.

4. The Ontario bait should be tested in New York.

5. Different markers should be used for different parts of the bait in order to be able to evaluate both the matrix and the vaccine carriers.

6. A group initiative is needed to make it feasible to analyze serum samples for protein-bound iodine (after use of iophenoxic acid in baits).

7. The results of laboratory efficacy tests should be interpreted with caution when baits are to be evaluated for field use.

8. Baiting stations could be used to attract raccoons to central points, especially in urban areas.

9. In order to evaluate distribution methods it will be necessary to test broad types of systems, including aerial distribution. It will be difficult to study large areas, however, due to limitations of sample returns and budgets.

10. Tests are needed to determine the optimum and most economic bait densities.

C. Surveillance

1. An early warning system is needed for rabies outbreaks, preferably one that uses active surveillance; this may include the use of roadkills or dead animals submitted by game wardens. Any positives diagnosed by this system should be included in the regular reporting network.

2. In order to pinpoint the exact location of cases a UTMC or other objective grid system should be used, especially where areas have been baited for rabies control by states. When those cases are plotted attempts should be made to relate them with habitat.

3. A lead U.S. agency should be responsible for overall wildlife rabies control programs.

MONGOOSES Vargas, Lord, Nettles, Niu

A. Population Estimates

1. Although presently available data indicate rather dense mongoose populations averaging about 5 mongooses per hectare, further population investigations should be carried out on different Caribbean islands using newer techniques and trap placement strategies.

2. Variants of the mark/recapture method are probably the most applicable for such studies, but a simultaneous cross-checking variant of the removal method would add credence to those results.

3. Those population estimates should be accompanied by a standard line of tracking boards (ten meters between stations) placed in the study area. This technique only provides an index of relative abundance, but if repeated enough times would probably make it possible to relate tracking board indexing to density measured by mark/recapture and removal techniques.

4. Because the tracking board method is so much less costly (traps are both expensive and readily stolen) they could be extended to different habitat types to produce estimates for many parts of the country. These results could then be extrapolated to land use maps to provide crude estimates of mongoose population density throughout a large geographic area.

5. There is a need for radio telemetry studies to determine home range size and movements.

B. Baits

1. We still cannot say that we have a bait for mongooses. We have a ' candidate' bait that must be field tested to compare it with other baits; for example, the fishmeal polymer bait in different formulations of hardness compared to the Canadian bait.

2. When a suitable bait for mongooses is found it should be tested with biological markers. Prior to that, of course, population estimates must be determined.

3. We have to assess just how many baits are needed per area for field placement.

C. Surveillance

1. Present passive surveillance of mongoose rabies is inadequate to provide the necessary information to control rabies by population reduction or oral vaccination, but it is not presently possible to increase diagnostic laboratories in the Caribbean because of limited financial resources.

2. In some countries it should be possible to take advantage of road kills to add information on rabies cases.

3. Available data needs to be compiled in map form to permit possible correlation with land use.

4. Specific surveillance of small rabies foci through interviews of families living in certain areas along with trapping mongooses for virus and antibody determinations should provide additional data to guide future control projects.

5. Data from both passive surveillance and future active efforts should be collected on a geographic scale detailed enough to permit determination of possible migratory outbreaks.

DOGS - Schneider, Flores-Collins, Fishbein, Belotto, Garza, Rodriguez-Dominguez, Hanlon, Vicens

A. Population Estimates -

1. A method has to be developed to quickly estimate the population of owned dogs in an area, such as village, town, or other geographic area. This may perhaps be done by counting the number of houses, then determining the estimated number of humans in the houses and the human: canine ratio by taking the ratio from previous census or rabies vaccination campaign data.

2. The WHO EPI (Expanded Program of Immunization) methodology may be useful to this end, including the use of cluster sampling. The goal is to obtain a formula for the ratio people: houses: owned dogs, and eventually also estimate the number of unowned dogs. One approach to this would be to mark the dogs in a mass vaccination campaign and calculate a Lincoln index on the ratio of owned to unowned dogs.

3. In all these estimates the approach in villages may differ significantly from that in large cities.

B. Baits

1. It is important to find a method for community distribution of baits by local personnel and health education workers; those persons would also prepare the community for such efforts.

2. With the distribution of baits one could then estimate the proportion of dogs that can be reached with baits.

3. The interaction of dogs, humans, and other animals with baits should be studied on a controlled basis before and after bait distribution.

C. Surveillance

1. Rabies surveillance should be incorporated into the normal health system surveillance, but a move should be made from passive (routine) surveillance to more active surveillance of rabies cases; this will require more facilities for diagnosis. The existing system will then also have to be better organized, including the routine investigation and control of outbreaks.

2. Emphasis should be placed on community participation and health education.

3. A minimum number of specimens submitted for diagnosis should be estimated, most likely 0.1 - 0.2% of the canine population per year.

4. The surveillance established should obviously be incorporated into regional, national, and international reporting systems.