ACCEPTANCE OF BAITS FOR DELIVERY OF ORAL RABIES VACCINE TO RACCOONS

Authors: Richard C. Rosatte, and Kenneth F. Lawson

Source: Journal of Wildlife Diseases, 37(4) : 730-739

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-37.4.730

Journal of Wildlife Diseases, 37(4), 2001, pp. 730–739

ACCEPTANCE OF BAITS FOR DELIVERY OF ORAL RABIES VACCINE TO RACCOONS

Richard C. Rosatte[1,3] **and Kenneth F. Lawson**[2]

[1] Ontario Ministry of Natural Resources, Wildlife Research and Development Section, Rabies Research and Development Unit, Trent University, Science Complex, P.O. Box 4840, Peterborough, Ontario K9J 8N8, Canada
[2] P.O. Box 121, King City, Ontario L7B 1A4, Canada
[3] Corresponding author (e-mail rick.rosatte@mnr.gov.on.ca)

ABSTRACT: During 1993–96 the Ontario Ministry of Natural Resources (Canada) implemented a research project to evaluate the efficacy of three candidate baits to deliver oral rabies vaccine to wild raccoons (*Procyon lotor*). Extensive field testing revealed that raccoon acceptance of Sugar-Vanilla baits (SV) at densities of 200/km^2 and 400/km^2, hand-placed in urban habitats of Scarborough (Ontario) during 1993, was 74% and 82%, respectively. Raccoon density in those areas averaged 11/km^2. Aerial placement of SV baits in rural habitats in Barrie (Ontario) during 1993 and 1994, yielded raccoon acceptance levels of 58% with a density of 100 baits/km^2, 59% at 75 baits/km^2, and 47% at 50 baits/km^2. Raccoon acceptance of SV baits was significantly lower in areas baited at the density of 50/km^2. Acceptance of Cheese baits (CH) at a density of 75 baits/km^2 was 52%. During 1996 trials in Barrie, modified SV baits with blister packs protruding through the matrix yielded raccoon acceptance values of 51% at a bait density of 54/km^2, whereas acceptance of regular SV baits was 39% at a density of 51 baits/km^2. Pooling of bait acceptance data for all years revealed that bait acceptance was highest for adult male raccoons. Raccoon density in rural habitats (Barrie, Ontario) where the studies took place, averaged 11–13/km^2. Puncture and impact testing of blister packs in baits suggested that they would adequately serve as a vehicle to contain oral rabies vaccine for delivery to raccoons via baits.

Key words: Bait acceptance, baits, biomarker, oral vaccination, rabies control, raccoon rabies, raccoons, tetracycline.

INTRODUCTORY COMMENTS

As raccoon rabies progressed towards Ontario (Canada) during the 1990's (Jenkins and Winkler, 1987; Rosatte et al., 1993; Rosatte et al., 2001) the Ontario Ministry of Natural Resources (O.M.N.R.) initiated a research project to develop baits to deliver oral rabies vaccine to raccoons. This proactive approach towards bait development was advantageous as raccoon rabies entered Ontario from New York State in July 1999 with 51 cases being reported as of April 2001 (Wandeler and Salsberg, 1999; Rosatte et al., 2001). If the disease becomes enzootic over large geographic areas of the Province, an effective vaccine bait will be the most cost effective means to control raccoon rabies (Rosatte et al., 2001).

During 1990–93, acceptance tests on captive and free-ranging raccoons revealed two candidate baits (Rosatte et al., 1998). Those baits, called Sugar-Vanilla (SV) and Cheese (CH) underwent further screening using radio-transmitters in baits to determine if there was a preference for either bait type by wild raccoons (Rosatte et al., 1998). This paper deals with the second phase of that research—determination of raccoon acceptance of the SV and CH baits at different densities in urban and rural habitats of southern Ontario using aerial and hand-distribution techniques (Bachmann et al., 1990) during 1993–96. Experiments also were initiated to evaluate the effectiveness of vaccine containers to deliver liquid vaccine to raccoons.

1993 urban baiting trial with Sugar-Vanilla baits in metropolitan Toronto

During 5 to 6 August 1993, 7,200 SV baits (hand manufactured and each containing 100 mg of tetracycline hydrochloride, an oral biomarker, in the bait matrix) (Fig. 1) were hand-placed in six 4 km^2 plots (3 plots at 400 baits/km^2 and 3 plots at 200 baits/km^2) in Scarborough (Ontario; 43°42′N, 79°25′W). The small plot size was justified for this experiment as rac-

FIGURE 1. Photograph (from top to bottom) of a Sugar Vanilla bait (SV), a cheese bait (CH), a small Sugar Vanilla bait (Sml SV), an SV and a sml SV bait (showing the difference in thickness of baits) and flocked blister packs that contain liquid vaccine.

coons in urban habitats are very sedentary (raccoon movements would not bias the results). One to 3 wk post baiting, raccoons were live-captured (Tomahawk model 106 and 108; Tomahawk Live-Trap Co., Tomahawk, Wisconsin, USA), immobilized (20–30 mg ketamine hydrochloride), eartagged for identification, vaccinated against rabies with a 1 ml intramuscular injection of Imrab inactivated rabies vaccine (Rhone-Merieux, Athens, Georgia, USA), a first premolar tooth collected, and released at the point of capture. The premolar teeth were then ground into 100 micron sections, mounted on microscope slides and observed for tetracycline fluorescence using ultraviolet light and a compound microscope (Johnston et al., 1987). A positive fluorescence indicated that the raccoon had consumed at least one SV bait. This allowed us to calculate the percentage of the raccoon population that contacted baits by dividing the number of tetracycline positive raccoons by the total number of raccoons in the sample.

1993 rural baiting trial with Sugar-Vanilla baits in Barrie, Ontario

On 4 August 1993, 22,547 SV baits (hand-manufactured) (Fig. 1) were aerially distributed in an approximate 300 km^2 area between Barrie and Bradford (Ontario; 44°10′N, 79°30′W). The baits contained 100 mg of tetracycline hydrochloride in the bait matrix as a biomarker to determine the percentage of raccoons consuming baits and to assess the potential of this bait to deliver oral rabies vaccine to raccoons. The baits were loaded for aerial distribution into the aircraft (a Turbo Beaver from the O.M.N.R. Sudbury Aviation Services Unit) in tubs (5 per flight) that contained about 1500 baits each.

The baiting area was divided into two 147 km^2 plots, one on either side of Hwy 400 between Barrie and Bradford. The plot (Cell A) west of Hwy 400 was baited at a density of 50 baits/km^2 and the plot (Cell B) east of Hwy 400 was baited at a density 100 baits/km^2. Each 147 km^2 cell was divided into 30 flight lines-the majority followed the east-west concession roads. The pilot attempted to keep the aircraft at a ground-speed of about 85 m.p.h. (136 km/hr) and an altitude of about 500 feet (152 m).

The total area was baited during three flights on 4 August 1993. The aircraft crew consisted of a pilot, navigator and two baiters. The two baiters sat facing the rear of the aircraft and tossed baits into a modified fish funnel that allowed the baits to

exit the bottom of the aircraft without hitting the fuselage. The baiting rate was determined with a metronome that emitted a visual and audible pulse corresponding with the desired baiting rate. Connected to the metronome was a "panic" button that allowed the navigator to signal the baiters to either start or stop baiting. The baiters were signaled to stop baiting when the aircraft approached major highways, vehicles, buildings, villages, etc.

Cell B (100 baits/km^2) was baited during the first two flights. Flight 1 consisted of 16 flight lines (7 km in length each and about .75 km apart), each lasting about 3 min. As the target bait density was 100/km^2, the baiters were required to drop about 500 baits per flight line. That density necessitated that one baiter drop two baits/second and the other one bait per second. This turned out to be close to the maximum rate that two baiters could easily handle (allowing time between flight lines to retrieve bait-trays from the tubs and rest their baiting arms). Flight 2 was a duplicate of flight 1 except there were 14 bait lines. In total, 15,046 baits were dropped into Cell B {(Flight 1—7,969 baits) (Flight 2—7,077 baits)} at a mean density of 102.4 baits/km^2. Flights 1 and 2 were each about 90 minutes in duration.

The lower bait density in Cell A (50/km^2) allowed the entire cell to be baited during one flight; however, the flight was 2 hrs in duration. To attain the desired bait density (250 baits per flight line), each baiter dropped one bait every 1.5 sec. In total, 7,501 baits were dropped over Cell A during flight 3. This equates to a mean bait density of 51.0/km^2.

Between 9 August and 17 September 1993, live-traps (Tomahawk model 106 and 108) were set in six randomly selected plots, each 3 km × 3 km in size within the baiting area to capture a sample of raccoons for estimating the percentage of the raccoons that contacted the SV baits. One hundred baited (sardines) live-traps were set for eight nights (4 consecutive nights/week) in each of the 9 km^2 plots. All captured raccoons were processed as in the Scarborough study.

1994 rural baiting trial with Sugar-Vanilla and Cheese baits in Barrie, Ontario

During the 1993 experiments, SV baits distributed aerially at a density of 100/km^2 elicited good acceptance in raccoons. Speculating that 100 baits/km^2 was more than adequate, we decided to distribute SV baits at a density of 75/km^2 during 1994, in an attempt to determine the minimum number of baits required to reach a substantial portion (i.e., 60%) of the raccoon population. Along with the SV baits, we decided to test Cheese (CH) baits (Fig. 1) which had proven to be attractive to raccoons during previous experiments (Rosatte et al., 1998). The composition of both baits was very similar to the traditional Ontario "fox bait" (Bachmann et al., 1990) except that different attractants were added to the matrix (Rosatte et al., 1998).

The 1994 study site was similar to the 1993 experiment—an approximate 300 km^2 area, divided into two 147 km^2 cells, located between Barrie and Bradford. About 11,250 SV baits were distributed in one of the 147 km^2 plots at a density of 75/km^2. A total of 10,768 CH baits were distributed in the other plot. Each of the baits contained a blister pack holding 1.8 ml of Oxymycine LP (180 mg of oxytetracycline hydrochloride) (Ayerst Laboratories, Guelph, Ontario) which served as an alternate biomarker to determine bait acceptance by raccoons (during 1993, the biomarker tetracycline HCl was in the bait matrix). A Turbo Beaver aircraft with a pilot, navigator and two baiters was used to distribute the baits on 11 July 1994. Three flights of about 1.5 hr each were required to distribute the baits. Aircraft speed, altitude, and flight line spacing were the same as during the 1993 experiment. Raccoons were live-captured and processed (as in 1993) in six plots (each 9 km^2 in size) located throughout the baited area (3 in each 147 km^2 cell).

1996 rural baiting trial with Sugar-Vanilla baits in Barrie, Ontario

During July 1996, Ayerst Laboratories (Guelph, Ontario) manufactured about 21,216 SV baits, each containing a blister pack (2 ml) and 100 mg (50 mg/ml) of oxytetracycline hydrochloride (Oxymycine LP). About one-half of the baits (10,357) contained blister packs made of flocked PVC material (a coating of material to which the bait matrix effectively adheres to) and protruded through the bait matrix (i.e., the top of the blister or lid was visible making it necessary to only have 1 precautionary label) (Fig. 1). As well, these blister packs had lid material that was more durable than the regular blister packs and the baits had less matrix material than the regular SV baits. These small SV baits were about 90% the size of the regular SV baits. The remaining baits (10,859) contained unflocked PVC blister packs with regular lids, that were embedded in the centre of the bait matrix (i.e., the blister pack was not visible). On 25 July 1996, baits were aerially dropped in two 147 km^2 plots located near Barrie (Ontario). One cell (to the east of HWY 400) was baited with baits containing the protruding flocked blister packs (PVC) while the other cell was baited with regular unflocked PVC blister pack baits. Attempts were made to bait both cells at a density of approximately 60 SV baits/km^2 with flight line spacing in both plots being 0.75 km. A Turbo Beaver aircraft was used to bait the plots with speed and altitude the same as during the 1993 experiment. Each of the two cells consisted of 30 flight lines, each being 7 km in length (about 3 min/line). That required that each of the two baiters dropped baits at a rate of 1 bait every 1.25 seconds. It took two flights (2 hrs 10 min/flight including about 40 minutes for ferrying time) to distribute the 15,473 baits.

Between 12–30 August two trapping teams placed 100 live-traps in each of six randomly selected 3 × 3 km cells in the baited plots. Each area was live-trapped over a 1 wk period (4 consecutive nights) in an effort to capture 20 raccoons in each cell. All captured animals were transported to the Midhurst Animal Compound (Ontario, Canada), immobilized (30 mg/kg Ketaset), and then euthanized with T-61 (2 ml intracardiac injection) (Hoechst Roussel Vet, Regina, Saskatchewan, Canada). Each raccoon had one canine, one 1st premolar, one 2nd premolar and a sample of bone collected from the jaw. Thin sections (100 μm) were prepared (isomet saw or by sanding with emery paper) from those tissues and mounted on microscope slides (Johnston et al., 1987). Bait acceptance by raccoons was determined by the presence of tetracycline in tooth sections and bone as in the 1993 study.

Vaccine container trial examining the efficacy of two types of blister packs to deliver liquid to raccoons

In an effort to develop an effective vehicle to deliver liquid rabies vaccine to raccoons, we tested two types of blister packs (PVC and Polystyrene) (Fig. 1) on wild raccoons (we initially screened seven types to arrive at the two tested). As the SV bait has been shown to be attractive to raccoons, the blister packs were offered to raccoons while encased in the matrix of the SV baits. Baits containing the blister packs and coloured water were offered to wild raccoons in Rosedale (a suburb of Toronto) (43°42′N, 79°25′W) and in King Township (30 km North of Toronto), Ontario (44°5′N, 79°10′W). Vaccine container effectiveness was determined by the number of perforations in the lid and the amount of liquid remaining in the container after the bait was consumed by a raccoon.

To estimate how sturdy the SV baits were, impact tests were carried out both in Barrie using a Turbo Beaver aircraft and in Bracebridge using a helicopter. About 100 flocked PVC blister baits with the blister packs protruding through the matrix (each containing 1.8 ml of tetracycline in solution) were dropped on the airport runway (pavement) and into a field at 85 mph

TABLE 1. Prevalence of tetracycline in the premolar teeth of raccoons sampled from plots in Scarborough, Ontario (urban habitat), that had been baited with Sugar-Vanilla baits during 5 to 6 August 1993.[a]

Study plots (*n*)	Bait density (baits/km^2)	Trapping dates	% Raccoons Tetracycline + (*n*)	% Adults Tetracycline + (*n*)	% Juveniles Tetracycline + (*n*)
3	400	9–27 Aug	82 (59/72)	82 (28/34)	74 (31/42)
3	200	8–27 Aug	74 (40/54)	86 (25/29)	60 (15/25)

[a] Mean raccoon density in the sampled areas was 11/km^2.

at an altitude of 300 to 500 ft. using the Turbo Beaver. About 500 of these baits were air-dropped from a helicopter in Bracebridge onto a variety of surfaces (pavement, gravel road, sand quarry, stubble fields).

1993 urban baiting trial with Sugar-Vanilla baits in the Metropolitan Toronto

During 5 to 6 August, 1993, 7,200 SV baits were hand-placed in six 4 km^2 plots in Scarborough (Ontario) where mean raccoon density was 11.3/km^2 (10.5–12.1, 95% CI). An average of 82% (59/72) of the raccoons sampled from areas baited at a density of 400/km^2 had consumed at least one SV bait as evidenced by the presence of tetracycline in their premolar teeth (Table 1). In areas baited at a density of 200/km^2, a mean of 74% (40/54) of the sampled raccoons had consumed baits (Table 1). However, we could detect no difference in raccoon acceptance of SV baits baited at a density of 400/km^2 versus 200/km^2 ($P > 0.05$).

We could detect no differences in the prevalence of tetracycline in adults (82%) versus juveniles (74%) in the raccoons that were sampled from areas baited at a density of 400/km^2 ($P > 0.05$) (Table 1). However, in areas baited at a density of 200 baits/km^2, adults had a higher prevalence of tetracycline (86%) than juveniles (60%) ($P < 0.05$) (Table 1).

A density of 200 baits/km^2 should be sufficient to reach at least 60% of the raccoons in urban Ontario habitat. That assumes that mean raccoon density is <20/km^2 (Rosatte et al., 1992; Rosatte, 2000) (i.e., higher raccoon density may require more baits).

1993 rural baiting trial with Sugar-Vanilla baits (biomarker in matrix) in Barrie, Ontario

A total of 416 different raccoons (541 total captures) were captured from the six plots in the baited area during 9 August–17 September 1993. However, premolar teeth were successfully extracted from only 378 raccoons. The estimated population density of raccoons (using a modified Petersen Index and capture-recapture data; Begon, 1979) in the baited area averaged 12.8 raccoons/km^2 (10.7–14.7, 95% CI). The sample consisted of 63% adults and 37% juveniles.

Based on the prevalence of tetracycline in the premolar teeth of captured raccoons an average of 58% (121/209) of the raccoons in the area baited at a density of 100 baits/km^2 consumed at least one SV bait (Table 2). Significantly fewer ($P < 0.05$) raccoons {47% (79/169)} contacted baits in the area baited at a density of 50 baits/km^2 (Table 2). At this density, males had higher acceptance values than females ($P < 0.0011$), however, we could not detect any differences between age and sex of raccoons and bait acceptance at a density of 100 baits/km^2.

We found a relationship between the trapping date and the percent of the raccoons that consumed baits (Figs. 2, 3). That is, raccoons captured 1 wk post baiting did not have as much time to find baits than those captured 2 to 4 wk post baiting, resulting in an underestimation of the number of raccoons consuming baits. Bait acceptance increased dramatically (from about 50% to about 80%) for raccoons collected 8 to 15 days post baiting ($R^2 = 0.69$, $P < 0.05$) (Fig. 2). It appears that about 2

TABLE 2. Comparison of bait acceptance by raccoons for combined bait types during 1993, 1994, and 1996 in the Barrie study areas with mean raccoon density between 11.0–13.0/km^2.

	% Acceptance			
Year	1993	1993	1994	1996
Bait type	SV[a]	SV[a]	SV/CH[a]	SV/sml SV[a]
Bait density/km^2	50	100	75	53
All sex/age classes	47.3	58.4	58.0	45.0
Juveniles	38.7	59.1	47.1	41.7
Adults	52.3	58.0	64.6	46.4
Males	61.0	63.1	60.6	57.4
Females	35.9	53.8	56.1	32.2
Juvenile males	53.3	60.5	43.6	57.1
Juvenile females	25.0	57.1	50.0	31.8
Adult males	66.0	64.6	72.0	57.4
Adult females	41.7	52.6	59.5	32.4

[a] SV = Sugar-Vanilla bait, CH = Cheese bait, small SV = small Sugar-Vanilla bait with the blister pack protruding through the matrix.

wk is required for raccoons to find and consume the baits. However, bait acceptance did not increase significantly 3 to 7 weeks post baiting (at least at a baiting density of 100 or 50/km^2) ($R^2 = 0.22$, $P < 0.15$) (Fig. 3).

We could detect no difference in the percentage of adults (55%) or juveniles (49%) which were positive for tetracycline ($P > 0.05$) (Table 2). That is, we could detect nothing to suggest that there was a difference in the ability of adults or juveniles to absorb tetracycline in their tissues during this time of year.

1994 rural baiting trial with Sugar-Vanilla and Cheese baits (biomarker in the blister pack) in Barrie, Ontario

Between 18 July and 26 August 1994, 437 raccoons (390 different individuals but teeth were extracted from only 381) were captured in the 300 km^2 study area. Mean raccoon density in those cells was 11/km^2 (9–12, 95% CI). Acceptance of SV baits (at a density of 75/km^2) by raccoons, (as indicated by tetracycline fluorescence in premolar teeth), was higher (59%, 116/197), though not statistically significant, than acceptance of CH baits (52%, 95/184) ($P < 0.25$). As there were no apparent sta-

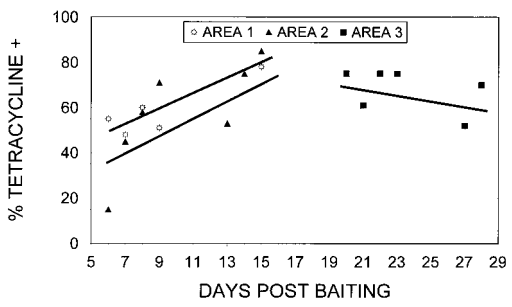

FIGURE 2. Percent of raccoons sampled from three study plots (Barrie) baited at a density of 100 baits/km^2 that were positive for tetracycline during 1993 over time.

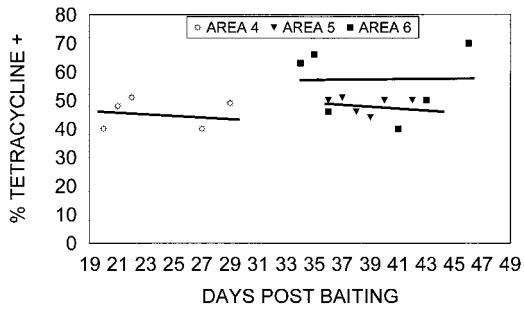

FIGURE 3. Percent of raccoons sampled from three study plots baited at a density of 50 baits/km^2 that were positive for tetracycline during 1993 over time.

TABLE 3. Bait acceptance by raccoons as determined by the presence of tetracycline in canine teeth in areas that were baited with small Sugar-Vanilla (sml SV) baits and regular SV baits in the 1996 Barrie study area.

Sex/Age	Small SV baits % Tetracycline +	Regular SV baits % Tetracycline +
Juveniles males	71 (5/7)	43 (3/7)
Juvenile females	50 (6/12)	10 (1/10)
Adult males	67 (16/24)	48 (11/23)
Adult females	22 (4/18)	42 (8/19)
Totals	51 (31/61)	39 (23/59)

tistical differences in acceptance between baits, the data for both baits were pooled. For pooled data, raccoon acceptance of both bait types was significantly greater for adults (65%) than juveniles (47%) ($P < 0.001$) (Table 2).

1996 rural baiting trial with Sugar-Vanilla (2 sizes) in Barrie, Ontario

Attempts were made to bait at a density of about 60/km^2. However, due to strong winds and the resultant fluctuating speed (70–90 knots) of the aircraft, actual bait density was lower. In the cell east of Hwy 400, 7,994 flocked blister S-V baits were distributed (210 baits/tray, 1700 baits/container). Actual bait density was 54.4 baits/km^2. The crew dropped 7,479 baits (regular unflocked PVC blisters) in the cell to the west of Hwy 400. That resulted in a baiting density of 50.9/km^2.

A total of 120 raccoons were captured between 12 to 30 August 1996 in the 300 km^2 bait drop area south of Barrie. The canine teeth from these animals were examined for the presence of tetracycline. Overall acceptance by all sex and age classes of raccoons in the three trapping cells that were baited with the small flocked blister (PVC) S-V baits was 51% (31/61) (Table 3). That was not statistically different from raccoon acceptance {39% (23/59)} in cells baited with the regular PVC blister SV baits (unflocked) (Table 3) ($P < 0.192$). However, when individual groups were considered, juveniles and juvenile females had significantly higher acceptance values for the flocked small PVC SV bait compared to the regular PVC SV bait ($P < 0.05$). Although data are lacking, that may be a function of younger raccoons with smaller mouths having a harder time eating the regular sized bait compared to the smaller bait. When data for both bait types were pooled, bait acceptance by male raccoons was significantly greater (57%) than acceptance by females (32%) for both bait types (Table 2) ($P < 0.005$). Although unproven, this may be a function of male raccoons traveling more extensively than females.

The modified SV experimental bait (flocked PVC blister protruding through the bait matrix) (Fig. 1) showed great promise to economically deliver oral rabies vaccine to raccoons for a number of reasons. Acceptance at a bait density of 54/km^2 was 51%, therefore higher baiting densities with this bait should result in bait acceptance levels exceeding 60%. Most importantly, the modified bait only requires one label and is comprised of less matrix material i.e., it is cheaper to make and is smaller than the regular SV bait (greater aircraft payload).

Our long-term goal is to produce sufficient data to be able to predict with confidence, the most cost-effective bait density to vaccinate a substantial portion (about 60%) of a raccoon population in urban and rural habitats of Ontario.

Comparison of 1993, 1994 and 1996 Barrie trials

During all years, adult raccoons had higher acceptances ($P < 0.05$) of all bait types than juveniles at densities of < 100 baits/km^2 when the data were pooled on a yearly basis (Table 2). As well, males had higher acceptance at bait densities < 75/km^2 ($P < 0.05$). In fact, adult males had the highest acceptance rates for combined years with a maximum acceptance rate of 72% during the 1994 baiting program (Table 2). However, the targeting of a particular age/sex cohort of the raccoon popu-

TABLE 4. Percentage of blister packs (PVC[a] and Polystyrene[b]) offered to wild raccoons that had perforations in the plastic and lids and >50% of the liquid missing.

Area of trial	% of Blisters with perforations in the lids (*n*)		% of Blisters with perforations in the plastic		% of Blisters with >50% of liquid missing	
	PVC	Polyst	PVC	Polyst	PVC	Polyst
King Tsp	91 (50/55)	78 (43/55)	20 (11/55)	67 (37/55)	87 (48/55)	96 (53/55)
Rosedale	100 (17/17)	100 (16/16)	0 (0/17)	100 (16/16)	100 (17/17)	100 (16/16)
Total	93 (67/72)	83 (59/71)	15 (11/72)	75 (53/71)	90 (65/72)	97 (69/71)

[a] PVC = polyvinyl chloride blister pack, Polysty = polystyrene blister.
[b] Blister packs were contained in Sugar-Vanilla baits.

lation to improve bait acceptance would not be realistic or cost-effective.

Vaccine container trial examing efficacy of blister packs

As the Sugar-Vanilla bait matrix showed great promise for raccoons, we tested two types of vaccine containers to examine their potential to deliver liquid rabies vaccine to wild raccoons. During August and November 1993, SV baits containing blister packs (each with 2.0 ml of water) in the baits, were offered to wild raccoons in both urban and rural habitats. Eighty-five percent (79/99) of the blister packs in SV baits offered to wild raccoons in suburban/rural habitat were punctured and had 50% or more of the liquid missing. As well, all (38/38) of the SV baits contacted by 17 different raccoons in urban habitat were completely eaten and all of the blister packs (19 normal and 19 inverted blisters) were well punctured and had no or very little fluid remaining.

Greater numbers of polystyrene blister packs (75%) had raccoon tooth perforations in the plastic than the PVC blister packs {(15%) ($P < 0.001$) (Table 4)}. In the Rosedale experiment the mean number of punctures in the polystyrene plastic was 3.38 (3.76 SD), while the PVC plastic was not punctured ($P < 0.001$). This is probably a function of the polystyrene material being more brittle than the PVC plastic. That is, when a raccoon bites the PVC blister, the plastic tends to bend while the polystyrene is more apt to fracture or puncture. Theoretically, use of polystyrene blisters should allow greater quantities of liquid vaccine in the blister pack to enter the oral cavity of the raccoon. However, we could detect no significant difference in the amount of liquid that was missing from either of the blister pack types {(90% PVC versus 97% Polystyrene) ($P < 0.10$) (Table 4)}. Unfortunately, during this experiment, there was no way of telling whether all of the liquid entered the oral cavity or was lost as spillage. We are currently working on an experiment to quantitatively measure the amount of liquid that raccoons ingest from the blister packs.

Impact tests to indicate the effects of impact on SV baits were carried out both in Barrie using the Turbo Beaver and in Bracebridge using a helicopter. Of the flocked PVC blister baits dropped at the Barrie airport, 73 in total were found. None were ruptured. Only one bait (one that landed on the paved runway) was leaking fluid as the top of the blister was curled due to hitting the pavement at 85 mph. Of the 500 flocked blister S-V baits dropped by chopper in Bracebridge, 327 were found. None were ruptured or leaking although some of the baits were severely damaged due to impacting on gravel and pavement. It appears the flocked PVC small SV bait is of sufficient integrity to

withstand the rigors of being aerially dropped in raccoon habitat. Although one vaccine container was found to be leaking in the Barrie experiment, baits are not normally dropped on hard surfaces such as pavement.

Bait density/raccoon acceptance curve

We now have good data on raccoon acceptance of SV baits at densities of 50, 75, and 100 baits/km^2. Ideally, a curve relating bait density and bait acceptance by raccoons would be a valuable tool in determining the most cost efficient density at which to bait raccoons for the control of rabies. At present, we believe that a density of about 75 baits/km^2 in rural habitats and 200 baits/km^2 in urban areas of southern Ontario, should be more than sufficient to reach a significant portion of a raccoon population. However, additional experimentation is needed to determine if fewer baits targeted to specific habitats and with specific spacing between flight lines will result in high bait acceptance by raccoons. This will have a significant impact on the cost of oral rabies vaccination of raccoons. Those experiments are in progress and results will be published in the future.

Cost to distribute Sugar-Vanilla or Cheese baits for raccoon rabies control

Currently, vaccinia-rabies glycoprotein (Raboral V-RG®) vaccine (Merial Limited, Athens, Georgia) (Rupprecht et al., 1986, 1988) is commercially available (in the U.S.A), however, it has yet to be licensed for use in wildlife in Canada. It has been used in an emergency situation to control an outbreak of raccoon rabies in eastern Ontario (Rosatte et al., 2001). At current prices (April, 2001), the cost to aerially distribute Sugar-Vanilla baits containing blister packs and V-RG vaccine at a density of 75/km^2 is about $200.00 Cdn/km^2 (Rosatte et al., 2001).

Sugar-Vanilla baits containing either Polystyrene or PVC blister packs, aerially dropped at a density of 75/km^2, with a flight line spacing of 0.75 km. should be an effective vehicle to deliver oral rabies vaccine to raccoons in rural Ontario habitats, assuming a mean raccoon density of 12/km^2 or less in rural habitats. Bait density in urban habitats of Ontario should be at least 200/km^2, assuming average raccoon densities are < 20/km^2. However, more research on raccoon movements and habitat utilization on a seasonal basis, as well as bait density and flight line spacing, the time of year to bait and number of times to bait annually, is needed to reduce the cost of oral vaccination of raccoons with baits. Research on effective oral vaccines for raccoons needs to be quickened so that the complete delivery system—bait, blister pack, vaccine, bait density, flight line spacing, time of year, etc., can be tested in the field before raccoon rabies becomes enzootic in Ontario. The cost of oral rabies vaccines also needs to be more competitive if oral vaccination is going to be a feasible option for the control of raccoon rabies over large geographic areas in Ontario, Canada.

ACKNOWLEDGMENTS

The O.M.N.R. Rabies Research Program was supported by the Rabies Advisory Committee, J. Carlson, Chairman, and by Dr. C. Davies, Manager, O.M.N.R., Wildlife Research and Development Section. The success of the raccoon rabies bait development programs would not have been possible without the dedication of the trappers, technicians and volunteers associated with the programs. They included: M. Power, D. Grieve, R. Warren, M. Wood, M. Allan, V. Gross, J. Carnie, J. Solly, D. Persaud, E. Wood, T. Archer, R. Phaneuf, L. Bruce, T. Davies, D. O'Leary, T. Etwell, A. James, D. Joachim, D. Wiedel, S. Miner, and A. Silver. Special thanks to N. Ayers and B. Turcott who piloted the Turbo Beaver aircraft during these experiments. Members of the O.M.N.R., Rabies Research and Development Unit provided input toward the design and implementation of the 1993–96 raccoon bait development programs including C. D. MacInnes, P. Bachmann, C. Nunan, and S. Taylor. D. Johnston, O.M.N.R., Rabies Research Unit, (retired) provided technical advice during the experiments. K. Ing and L. Browne analyzed the 1993–96 bait comparison data. C. D. Mac-

Innes reviewed and provided constructive comments on the manuscript. This is O.M.N.R., Wildlife Research and Development Section, Contribution No. 20.

LITERATURE CITED

BACHMANN, P., R. N. BRAMWELL, S. J. FRASER, D. A. GILMORE, D. H. JOHNSTON, K. F. LAWSON, C. D. MACINNES, F. O. MATEJKA, H. E. MILES, M. E. PEDDE, AND D. R. VOIGT. 1990. Wild carnivore acceptance of baits for delivery of liquid rabies vaccine. Journal of Wildlife Diseases 26: 486–501.

BEGON, M. 1979. Investigating animal abundance: Capture-recapture for biologists. University Park Press, Baltimore, Maryland, 97 pp.

JENKINS, S. R., AND W. G. WINKLER. 1987. Descriptive epidemiology from an epizootic of raccoon rabies in the middle Atlantic States, 1982–1983. American Journal of Epidemiology 126: 429–437.

JOHNSTON, D. H., D. JOACHIM, P. BACHMANN, K. KARDONG, R. STEWART, L. DIX, M. STRICKLAND, AND I. WATT. 1987. Aging furbearers using tooth structure and biomarkers. *In* Wild furbearer management and conservation in North America, M. Novak, J. Baker, M. Obbard, and B. Mallock (eds.). Ontario Trappers Association, North Bay, Ontario, pp. 228–243.

ROSATTE, R. C. 2000. Management of raccoons (*Procyon lotor*) in Ontario, Canada: Do human intervention and disease have significant impact on raccoon populations? Mammalia 64: 369–390.

———, M. J. POWER, C. D. MACINNES, AND J. B. CAMPBELL. 1992. Trap-vaccinate-release and oral vaccination for rabies control in urban skunks, raccoons and foxes. Journal of Wildlife Diseases 28: 562–571.

———, ———, ———, D. H. JOHNSTON, P. BACHMANN, C. P. NUNAN, C. WANNOP, M. PEDDE, AND L. C. CALDER. 1993. Tactics for the control of wildlife rabies in Ontario Canada. Scientific and Technical Reviews of the Office of International Epizootics 12: 95–98.

———, K. LAWSON, AND C. D. MACINNES. 1998. Development of baits to deliver oral rabies vaccine to raccoons in Ontario. Journal of Wildlife Diseases 34: 647–652.

———, D. DONOVAN, M. ALLAN, L. HOWES, A. SILVER, K. BENNETT, C. MACINNES, C. DAVIES, A. WANDELER, AND B. RADFORD. 2001. Emergency response to raccoon rabies introduction in Ontario. Journal of Wildlife Diseases 37: 265–279.

RUPPRECHT, C. E., A. N. HAMIR, D. H. JOHNSTON, AND H. KOPROWSKI. 1988. Efficacy of a vaccinia rabies glycoprotein recombinant virus vaccine in raccoons (*Procyon lotor*). Reviews of Infectious Diseases 10: S803–S809.

———, T. J. WIKTOR, D. H. JOHNSTON, A. N. HAMIR, B. DIETZSCHOLD, W. H. WUNNER, L. T. GLICKMAN, AND H. KOPROWSKI. 1986. Oral immunization and protection of raccoons (*Procyon lotor*) with a vaccinia rabies glycoprotein recombinant virus vaccine. Proceedings of the National Academy of Science U.S.A. 83: 7947–7950.

WANDELER, A., AND E. SALSBERG. 1999. Raccoon rabies in eastern Ontario. Canadian Veterinary Journal 40: 731.

Received for publication 17 November 1999.