Journal of Wildlife Diseases, 34(3), 1998, pp. 647–652

Development of Baits to Deliver Oral Rabies Vaccine to Raccoons in Ontario

Richard C. Rosatte,[1,3] Kenneth F. Lawson,[2] and Charles D. MacInnes,[1] [1] Ontario Ministry of Natural Resources, Wildlife and Natural Heritage Science Section, Rabies Research Unit, Trent University, P.O. Box 4840, Peterborough, Ontario, Canada, K9J 8N8; [2] P.O. Box 121, King City, Ontario, L7B 1A4; and [3] Corresponding Author: (e-mail: rosattri@epo.gov.on.ca).

ABSTRACT: During 1993, the Ontario Ministry of Natural Resources, Rabies Research Unit, conducted experiments to develop a bait that would be attractive to raccoons (*Procyon lotor*) and serve as a vehicle to deliver oral rabies vaccine to that species. Testing of six candidate baits on captive and wild raccoons revealed that the best baits in terms of attractiveness to raccoons were a sugar-vanilla bait and a cheese powder bait. Further testing of those two baits containing miniature radio-transmitters indicated there was no preference between the baits, with respect to acceptance by raccoons; however, as there were fewer problems in mass producing the sugar-vanilla bait, it was selected for larger scale experiments.

Key words: Bait acceptance, oral vaccination, *Procyon lotor*, raccoons, raccoon rabies, rabies controls.

Ontario (Canada) has taken a proactive approach to keep rabies from becoming established in raccoons (*Procyon lotor*) in this province (Rosatte et al., 1997). During 1994–97 buffer zones of vaccinated raccoons were created using a tactic called Trap-Vaccinate-Release (TVR) (Rosatte et al., 1992) at sites along the New York/Ontario border where the disease was expected to spread into Ontario. However, there are drawbacks when using TVR for the control of raccoon rabies. Foremost, it is very labor intensive and time consuming. It took five trapping teams 6 mo to TVR raccoons on a 680 km^2 area in Niagara Falls, Ontario (R. C. Rosatte et al., unpubl. data). If time and labor are critical factors, clearly TVR is not an acceptable candidate for rabies control. That is, if a previously rabies-free area is suddenly inundated with rabies cases over a large geographic area, it is doubtful TVR would be able to contain/eradicate the outbreak due to the length of time required to immunize a significant portion of the population. A much more feasible alternative to TVR for rabies control in large epidemic/endemic areas would be by aerial distribution of baits containing oral rabies vaccine (MacInnes, 1987, 1988; Rosatte et al., 1993).

Unfortunately, the use of oral immunization with baits for the control of raccoon rabies is not without problems. First, the bait (beef tallow with chicken/cod as attractants) (Bachmann et al., 1990) and vaccine (ERA) (Lawson et al., 1992) that is effective for rabies control in foxes (*Vulpes vulpes*) in Ontario, does not work very well in raccoons because of poor bait acceptance (5%–47%) and seroconversion (30%), at least at the densities effective for fox rabies control (Bachmann et al., 1990; Johnston et al., 1988; Lawson et al. 1989; Rosatte et al., 1990, 1992). However, much experimentation has been conducted in North America to find a bait and vaccine combination that is effective in producing rabies immunity in raccoons (Hadidian et al., 1989; Hanlon et al., 1989; Johnston et al., 1988; Linhart et al., 1991, 1994; Rupprecht et al., 1986; Wandeler, 1991). The effort to develop a bait to deliver oral rabies vaccine to raccoons in Ontario has been very intense and long term. We selected six bait types for extensive testing on captive animals. That experiment was designed to diminish the number of candidate baits down to two for an experiment in the wild with radio-transmitters. This paper details the efforts to develop baits to deliver oral rabies vaccine to raccoons in Ontario, Canada.

Baits were manufactured at the Ontario Ministry of Natural Resources (OMNR) Rabies Research Unit (Maple, Ontario, Canada). The basic formula for each 20 g

FIGURE 1. The basic physical appearance of the bait for oral rabies vaccination of raccoons to which attractants were added.

bait (3.5 × 3.5 × 1.5 cm in size) (Fig. 1) is outlined in Table 1. Attractants were added to the main ingredients of the baits which were oleo beef stock (Bakers and Us, Rexdale, Ontario, Canada), Microbond® wax (International Wax Ltd., Agincourt, Ontario, Canada) and mineral or vegetable oil (Daminco Inc., Mississauga, Ontario, Canada) (Table 1).

To manufacture 250 baits, 2.95 kg of oleo and 1.6 kg of microbond wax were melted in a 5,000 ml pot (85–95 C). Other ingredients such as vegetable/mineral oil and attractants were then added. The matrix was allowed to cool (75 C) and then poured into plastic bait moulds (20 baits/mould tray) (W. T. Lynch Foods, Scarborough, Ontario, Canada). Baits were placed in a refrigerator until they hardened, removed from the moulds, packaged, and stored at −20 C.

During 5–7 April 1993, six different bait types (Table 1) were offered in random order to each of 100 captive raccoons at the Midhurst Animal Compound (Midhurst, Ontario, Canada). Each raccoon was housed individually in a small pen (1m × 2m), and was offered one bait type at a time. An observer noted the time that was required for the raccoon to consume the bait and the percentage of the bait that was consumed during 30 min of observation. If after 30 min the bait had not been consumed, it was removed from the pen. At the end of the day any uneaten baits were returned to the pen and left overnight. The following morning the pen was checked to observe if the baits had been consumed.

During 13–14 April 1993, the six differ-

TABLE 1. Composition (%) of six types of baits used for raccoon acceptance studies.

Bait Type[a]	Oleo	Microbond[a] wax	Bees wax	Veg[b] oil	Cod oil	Attractant
Peanut butter	32	28	0	10	0	Peanut butter 30
Sugar-Vanilla	42	28	0	9	0	Icing sugar 20 Vanilla 1
Cheese	42	28	0	10	0	Cheese powder 20
Honey-bees wax	62	0	28	9	0	IFF[c] honey 1
Banana-bees wax	62	0	28	8	0	IFF[c] banana 2
Sea food-cod-oil	62	28	0	0	8	IFF[c] seafood 2

[a] 100 mg of tetracycline hydrochloride was added to each bait with each bait weighing 15–20 g.
[b] Veg = vegetable or mineral oil.
[c] IFF = International Flavours and Fragrances, Concorde, Ontario, Canada.

ent bait types (Table 1) were offered (two types at a time) to raccoons in large pens (3m × 5m) at the Midhurst animal facility. Two different bait types were placed in each pen and covered with debris. A single raccoon was introduced into the pen and observed for 20 min to determine which bait type was investigated/consumed first. If the baits were not consumed during that time, they were removed from the pen, but were returned at the end of the day to be left overnight. In total, 15 raccoons were used to compare the 15 different bait comparisons. Each bait type comparison was replicated three times over a 2 day period.

During the period 3 May to 11 June 1993, cheese and sugar-vanilla baits (Table 1) (Fig. 1) containing miniature radio-transmitters (Lotek Engineering, Newmarket, Ontario, Canada) were placed at a total of 18 different study sites consisting of nine urban and nine rural locations in southern Ontario. Transmitter-baits were located each morning using SRX-400 receivers (Lotek Engineering, Newmarket, Ontario, Canada) and four element Yagi antennae (Wildlife Materials Inc., Carbondale, Illinois, USA) to determine the percentage of the bait matrix that had been consumed during the previous night. The exact time that each transmitter-bait had been moved during the previous night also was recorded at six of the rural study sites using an automated radio telemetry tracking system (Lotek Engineering, Newmarket, Ontario, Canada). This procedure provided us with an estimate of bait selection by raccoons (i.e., did they chronologically select cheese over sugar-vanilla). During each baiting day, all transmitters were coated with new bait matrix material. The species of animal that contacted the baits was determined by observation and identification of tracks at the study site as well as by tooth impressions in the bait material.

The data collected during the captive animal experiments were initially analysed using a parametric analysis of variance (ANOVA) technique (Zar, 1974). An F test in the ANOVA was used to detect if raccoon response to the various bait types differed (Zar, 1974). A Ryan-Einot-Gabriel-Welsch Multiple F test was utilized to determine the order of preference of baits and to determine which bait types produced the best response times in raccoons (Siegel, 1956). Differences in acceptance of transmitter-baits was tested using Chi Square analysis (Zar, 1974).

During the small pen experiment, six different baits were offered to captive raccoons, one bait at a time (Table 1). Only about 20% of the raccoons responded to the baits when they were placed in their pens during the 30 min observation period. The low response rate was most likely a function of human observer presence, and the varying length of time the individual animals had been in captivity. That is, animals that had been in captivity longer were less intimidated by human presence. An analysis of variance of the investigation and consumption times for the different bait types and the number of raccoons responding to each bait type indicated that the response of raccoons to various baits differed ($P < 0.0001$, $F = 8.36$, Table 2). The order that any particular bait type was given to raccoons apparently did not affect the results ($P < 0.8106$, $F = 0.73$). However, raccoons that had been in captivity for several months, responded better to the baits than recently captured raccoons ($P < 0.0001$, $F = 4.06$). A Ryan-Einot-Welsch Multiple F test (Siegel, 1956) was used to determine which bait types produced the best response (lowest investigation and consumption times) from the raccoons. The sugar-vanilla, cheese, and sea-food baits produced the best response in raccoons with respect to investigation and consumption times ($P < 0.05$). However, there was no detectable difference in the performance of those three baits when tested against each other.

Including the baits left overnight in the pens, the order of raccoon bait consumption in terms of the percentage of individ-

TABLE 2. Investigation and consumption times for six different bait types offered to raccoons on 5–7 April 1993 during a small pen experiment at Midhurst, Ontario.

Bait type	Time to investigate bait (min : sec) mean (SD)[a] (n)[b]	Time to eat 50% of bait (min : sec) mean (SD) (n)	Time to eat 100% of bait (min : sec) mean (SD) (n)
Peanut butter	1:56 (3:40) (18)	7:28 (7:15) (12)	16:40 (9:59) (10)
Sugar-Vanilla	0:34 (:56) (18)	2:51 (2:30) (19)	6:12 (4:48) (17)
Cheese	1:11 (2:17) (25)	4:47 (6:48) (18)	8:04 (7:30) (16)
Honey-bees wax	1:53 (4:11) (21)	10:34 (9:15) (8)	18:00 (5:47) (4)
Banana-bees wax	2:03 (6:01) (21)	8:54 (8:29) (9)	20:31 (7:57) (7)
Sea-food	1:37 (3:06) (20)	2:15 (1:43) (11)	6:53 (8:06) (10)

[a] SD = standard deviation.
[b] n = number of raccoons out of 100 that investigated/consumed baits.

uals that consumed 100% of the bait type was cheese > sugar-vanilla > sea food > peanut butter > honey = banana (Fig. 2). However, statistically, there was no detectable difference in acceptance of the cheese, sugar-vanilla or the sea food baits ($P > 0.05$).

The order of bait preference in terms of which bait type was consumed first during the two choice bait comparison trial in the large arena was cheese > sugar-vanilla > honey > sea food > banana > peanut butter. However, there was no difference in acceptance between the cheese, sugar-vanilla and the honey baits ($P > 0.05$). The order of bait preference in terms of the percentage of raccoons which consumed 100% of the bait was cheese > sugar-vanilla > sea food > banana > peanut butter > honey (Fig. 2). However, there was no difference in acceptance between the cheese, sugar-vanilla and sea food baits ($P > 0.05$).

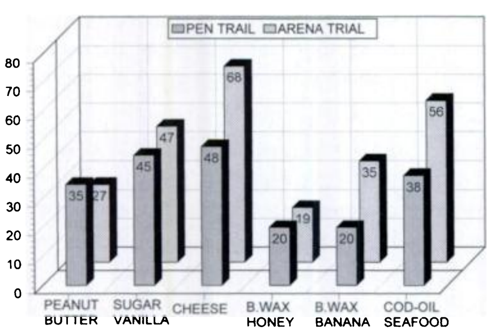

FIGURE 2. Percent of 100 captive raccoons at Midhurst, Ontario, that consumed 100% of a particular bait type during a small pen and a large arena experiment, 5–14 April 1993. Values are reflective of baits that were consumed during the day and night.

Forty-eight percent (69/144) of the transmitter-cheese baits were each 90 to 100% eaten by raccoons at the nine urban study sites (matrix not transmitters was consumed). As well, 54% (78/144) of the sugar-vanilla transmitter-baits were consumed by raccoons at the urban sites (no between bait differences in acceptance detected $P > 0.20$). More cheese and sugar-vanilla baits were eaten by raccoons at rural study sites than at urban sites ($P < 0.05$, Chi Square = 5.92). In fact, 76% (109/144), and 73% (105/144) of the cheese and sugar-vanilla baits were consumed by raccoons at rural study sites, respectively. However, no difference in acceptance was detected between those two bait types ($P > 0.05$). As well, we found

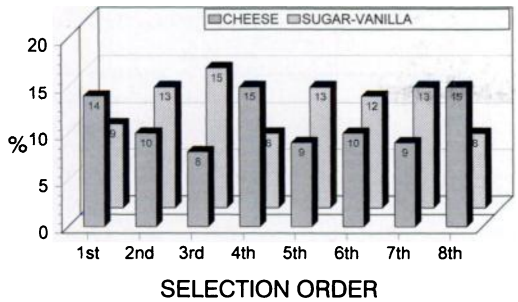

FIGURE 3. Selection order of eight transmitter baits by wild raccoons at six study sites during 3 May to 11 June 1993.

no evidence to suggest transmitters caused bait aversion in raccoons.

At each study site and during each baiting night, the number of cheese and sugar-vanilla baits that were completely eaten were compared. During each baiting night, a particular bait type was determined to have won the "bait acceptance contest" by raccoons, if acceptance was >25% higher than the other bait. We could detect no difference in acceptance with cheese winning 11 times and sugar-vanilla winning 10 times ($P > 0.05$).

Using the data collected from the radiotelemetry station at six rural sites, we determined the exact time that a particular bait type was moved by a raccoon. However, in terms of selection order of bait types we could not detect a preference for either bait type by raccoons in that cheese was selected first to fourth 47 times and sugar-vanilla was selected first to fourth 45 times ($P > 0.05$) (Fig. 3).

Experimentation with six candidate baits yielded two baits that should be effective to orally deliver liquid rabies vaccine to raccoons. Although we could detect no difference in preference of sugar-vanilla baits versus cheese baits, we concluded that there would be more problems with mass producing cheese baits as the material tends to coagulate and plug the machinery. This research provided us with guidelines for conducting larger scale experiments. We felt it was prudent to conduct additional field tests with the two baits to determine which would be the better candidate to deliver oral rabies vaccine to raccoons at specific bait densities over large geographic areas. The results of that research will be forthcoming.

The success of the raccoon bait development program would not have been possible without the dedication of many staff including M. Power, L. Calder, M. Pedde, M. Allan, D. Grieve, R. Warren, P. Bachmann, C. Nunan, D. Joachim, K. MacDonald and A. Silver. C. Nunan analysed the bait consumption time data. S. Taylor and S. Crosgrey assisted with the experiment at the Midhurst Animal Facility and D. Johnston, (retired), provided technical advice during the experiments. This is OMNR, Wildlife and Natural Heritage Science Section Contribution No. 97-01.

LITERATURE CITED

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

HABEL, C., A. HAMIR, D. SNYDER, R. JOYNER, J. FRENCH, V. NETTLES, C. HANLON, AND C. RUPPRECHT. 1992. Prerequisites for oral immunization of free-ranging raccoons (*Procyon lotor*) with a recombinant rabies virus vaccine: Study site ecology and bait system development. Journal of Wildlife Diseases 28: 64–79.

HADIDIAN, J., S. JENKINS, D. JOHNSTON, P. SAVARIE, V. NETTLES, D. MANSKI, AND G. BAER. 1989. Acceptance of simulated oral rabies vaccine baits by urban raccoons. Journal of Wildlife Diseases 25: 1–9.

HANLON, C., D. HAYES, A. HAMIR, D. SNYDER, S. JENKINS, C. HABLE, AND C. RUPPRECHT. 1989. Proposed field evaluation of a rabies recombinant vaccine for raccoons (*Procyon lotor*): Site selection, target species characteristics, and placebo baiting trial. Journal of Wildlife Diseases 25: 555–567.

JOHNSTON, D., D. VOIGT, C. MACINNES, P. BACHMANN, K. LAWSON, AND C. RUPPRECHT. 1988. An aerial baiting system for the distribution of attenuated recombinant rabies vaccine for foxes, raccoons, and skunks. Reviews of Infectious Diseases 10: S660–S664.

LAWSON, K., R. HERTLER, K. CHARLTON, J. CAMPBELL, AND A. RHODES. 1989. Safety and immunogenicity of ERA strain of rabies virus propagated in a BHK-21 cell line. Canadian Journal of Veterinary Research 53: 438–444.

———, H. CHIU, M. MATSON, P. BACHMANN, AND J. CAMPBELL. 1992. Studies on the efficacy and stability of a vaccine bait containing ERA® strain of rabies virus propagated in a BHK-21 cell line. Canadian Journal of Veterinary Research 56: 135–141.

LINHART, S., F. BLOM, G. DASCH, J. ROBERTS, R. ENGEMAN, J. ESPOSITO, J. SHADDOCK, AND G. BAER. 1991. Formulation and evaluation of baits for oral rabies vaccination of raccoons (*Procyon lotor*). Journal of Wildlife Diseases 27: 21–33.

———, ———, R. ENGEMEN, H. HILL, T. HON, D. HALL, AND J. SHADDOCK. 1994. A field evaluation of baits for delivering oral rabies vaccine to raccoons (*Procyon lotor*). Journal of Wildlife Diseases 30: 185–194.

MACINNES, C. 1987. Rabies. *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, Canada, pp. 910–929.

———. 1988. Control of rabies in wildlife: The Americas. *In* Rabies, J. B. Campbell and K. M. Charlton (eds.). Kluwer Academic Publishers, Boston, Massachusetts, pp. 381–405.

ROSATTE, R., M. POWER, AND C. MACINNES. 1990. Rabies control for urban foxes, skunks and raccoons. *In* Proceedings of the 14th Vertebrate Pest Conference, L. R. Davis and R. E. Marsh (eds.). The University of California, Davis, California, pp. 160–167.

———, ———, ———, AND J. 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. JOHNSTON, P. BACHMANN, C. NUNAN, C. WANNOP, M. PEDDE, AND L. 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.

———, C. MACINNES, R. TAYLOR WILLIAMS, AND O. WILLIAMS. 1997. A proactive prevention strategy for raccoon rabies in Ontario, Canada. Wildlife Society Bulletin 25: 110–116.

RUPPRECHT, C., T. WIKTOR, D. JOHNSTON, A. HAMIR, B. DIETZSCHOLD, W. WUNNER, L. 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.

SIEGEL, S. 1956. Non parametric statistics for the behavioural sciences. McGraw-Hill Book Company, New York, New York, 312 pp.

WANDELER, A. 1991. Oral immunization of wildlife. *In* The natural history of rabies. 2nd ed. G. M. Baer (ed.). CRC Press, Boca Raton, Florida, pp. 485–505.

ZAR, J. 1974. Biostatistical analysis. Prentice Hall Inc., Engelwood Cliffs, New Jersey, 620 pp.

Received for publication 5 August 1997.