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FIELD EVALUATION OF BAITS AND BAITING STRATEGIES FOR DELIVERING ORAL VACCINE TO MONGOOSES IN ANTIGUA, WEST INDIES

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ABSTRACT: A field study was conducted on Antigua, West Indies, to determine the feasibility of delivering an oral rabies vaccine or population control agent to free-ranging mongooses (*Herpestes javanicus*). Two biomarkers (tetracycline hydrochloride [THCL] and DuPont Oil Blue A® dye) and two bait types (DuPont polymer fish meal and polyurethane foam) were used to bait three study sites. Four hundred polymer baits containing both biomarkers were distributed at 36 central point bait stations (11 baits/station) on an 80 ha study site (5 baits/ha); 69% of the mongoose population consumed one or more baits. Two thousand baits containing THCL and 400 baits containing DuPont dye were distributed on two additional 100 ha study sites (24 baits/ha). Polymer fish meal baits were used on the first site and polyurethane baits on the second site. Based on the presence of biomarkers in bone or soft tissue, 96 to 97% of the mongooses at both sites consumed at least one bait. We conclude that oral baiting of mongooses is a feasible method for delivery of vaccines for the control of rabies in this species.

Key words: Oral vaccination, baiting, mongoose, *Herpestes javanicus*, biomarker, field study.

INTRODUCTION

The small Indian mongoose (*Herpestes javanicus*) is indigenous to Asia and has been introduced into South America, Hawaii (USA), and many of the Caribbean Islands (Nellis and Everard, 1983). Rabies is present in mongooses on the Caribbean Islands of Cuba, Puerto Rico, Grenada, and Hispaniola (Centers for Disease Control, 1985; Everard and Everard, 1988). Attempts to eliminate the disease by reducing the size of the mongoose population with strychnine baits have been unsuccessful (Nellis and Everard, 1983) and population reduction has had little lasting effect on the prevalence of the disease (Everard and Everard, 1988). Vaccination of wildlife by the conventional parenteral route is not practical due to the wide geographic distribution of infected populations and the difficulties associated with the capture and handling of large numbers of free-roaming wild animals. Hand vaccination is labor intensive, time consuming, and not considered cost effective (Animal and Plant Health Inspection Service, 1991). However, more recent trap-vaccinate-release studies targeting urban carnivore populations have been effective (Rosatte et al., 1992).

Rabies control by oral vaccination of red foxes (*Vulpes vulpes*) has been effective in a number of European countries (Schneider et al., 1988; Wandeler, 1988). Large scale oral vaccination trials for red foxes also have been conducted in Ontario, Canada (Johnston et al., 1988). Laboratory and field trials to orally vaccinate raccoons (*Procyon lotor*) have been undertaken in the eastern United States (Hadidian et al., 1989; Hable et al., 1991). However, there remains a need to demonstrate the feasibility of oral immunization for other wildlife hosts, including the mongoose (Wandeler, 1991).

The development of genetic recombinant vaccines has provided an effective means of vaccinating wildlife species such as raccoons that have proven difficult to orally immunize using attenuated rabies virus vaccines (Rupprecht et al., 1986; Blancou et al., 1989). However, only lim-

ited tests have evaluated the efficacy of the recombinant vaccines for mongooses (Esposito et al., 1992).

Baits for mongooses previously have been used to deliver toxins, particularly anticoagulants, for population reduction (Pimentel, 1955). More recently, M. C. Vargas (pers. comm.) and Linhart et al. (1993) conducted initial evaluations of mongoose baiting techniques and bait preferences in Puerto Rico and Antigua, West Indies.

As efforts to produce a safe, effective oral rabies vaccine for mongooses continue, concomitant work is needed to provide baits and baiting systems capable of delivering such a vaccine. Therefore, our objectives were to determine the effectiveness of tetracycline and DuPont Oil Blue A® dye as biological markers for mongooses; to compare the acceptance of two types of bait by mongooses; to determine the percentage of mongooses that can be administered baits; and to examine the effect of baiting density on bait acceptance rates by mongooses.

MATERIALS AND METHODS

Antigua is located along the outer edge of the Leeward Islands chain in the West Indies (17°6'N, 61°45'W) and is approximately 276 km² in size. A detailed account of its topography, vegetation, temperature, and rainfall patterns is provided by Harris (1965). Wildlife species present include only mongooses, introduced into Antigua from Jamaica in 1879 to control rats in the sugarcane plantations (Allen, 1911), Norway rats (*Rattus norvegicus*), black rats (*Rattus auropunctatus*), house mice (*Mus musculus*), and numerous species of birds.

Three study sites were chosen based on the presence of mongooses and other ongoing studies. These sites were designated as Blackmans (80 ha), Langfords (100 ha), and Bodkins (100 ha). Langfords is located 2.75 km north of the capital city of St. Johns. Blackmans is located 6 km to the SE and Bodkins is approximately 10 km S of St. Johns. The shortest straight line distance between any of the study areas is 7 km. Vegetation on the study sites was predominately acacias and grasses; the areas were used to graze cattle which either were tethered or allowed to roam the three sites freely.

Two biomarkers were administered to captive mongooses. Tetracycline hydrochloride (THCL) (Sigma Chemical Company, St. Louis, Missouri, USA), an antibiotic that is deposited in growing bone and teeth, can be detected by examination under ultraviolet (UV) light. It has been widely used as a biomarker for various species (Linhart and Kennelly, 1967; Savarie et al., 1992). DuPont Oil Blue A® dye (ICI Americas Inc., Wilmington, Delaware, USA) is a short term marker that we have used previously to mark mongooses (Linhart et al., 1993). It is retained in fatty tissues of the body.

Captive mongooses were orally administered THCL and DuPont dye prior to field trials to determine biomarker efficacy. Twenty-four mongooses were captured in live traps (15 × 15 × 60 cm, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) and housed in 38 × 38 × 38 cm wire cages constructed of 1.28 cm² hardware cloth. They were fed a locally purchased canned cat food and provided water ad libitum. Twelve mongooses, four males and eight females, were offered a DuPont bait containing 25 mg THCL and 12 mg DuPont dye. An additional 12 mongooses, two males and 10 females, were anesthetized using 8 mg/kg ketamine hydrochloride (Avecol Corporation, Ft. Dodge, Iowa, USA) and 2 mg/kg xylazine (Mobay Corporation, Animal Health Division, Shawnee, Kansas, USA) and given 25 mg THCL and 12 mg DuPont dye in 1 cc of corn oil via a Monoject® 10 FR stomach feeding tube (Sherwood Medical, St. Louis, Missouri). On days 1, 3, 6, and 12 post-ingestion, three mongooses from each group were euthanized and examined. All animals first were anesthetized using ketamine/xylazine, and then euthanized with 0.2 mg/kg of a pentabarbital euthanasia solution administered intracardially (Fatal Plus®, Vortech Pharmaceuticals, Dearborn, Michigan, USA). Abdominal, mandibular, femur marrow, and tail fat were examined for the robin's egg blue color characteristic of tissues marked by the dye (Linhart et al., 1993). Mandibles were removed, frozen, and later cross-sectioned at the second premolar using an Isomet® low speed, double bladed saw (Buehler Ltd., Lake Bluff, Illinois, USA). Acetate spacers were placed between the diamond Isomet® saw blades to produce sections 100 to 150 μm thick. Sections were mounted in glycerin on slides, covered with 0.17 mm cover slips, and stored in the dark at −4 C to minimize photodegradation of THCL-induced fluorescence (Buyske et al., 1960). With a UV-lighted microscope, sections were examined at 80× for the presence of fluorescence in or adjacent to Haversion canals and along the outer edge of bone cross sections (Jorch and Anderson, 1980).

A proprietary polymer-based bait (E.I. DuPont DeNemours and Company Inc., Or-

ange, Texas, USA) containing unspecified amounts of a waterproof polymer as a binder, and soybean oil and fish meal were used on two study areas (Blackmans, Trial 1; Bodkins, Trial 3). The extruded baits were cylindrical in shape and 25 × 12 mm in size, with a 4 mm hole through the center (Fig. 1). The polymer baits for Trial 1 contained both 25 mg of THCL and 12 mg of DuPont dye. Baits used in Trial 3 contained either 25 mg THCL or 12 mg of DuPont dye.

A polyurethane foam sleeve bait was distributed at Langfords (Trial 2). This bait originally was developed for raccoons (Linhart et al., 1991) and subsequently modified for mongooses (Linhart et al., 1993). Baits consisted of a 15 × 28 mm polyurethane foam sleeve with an 8 mm hole in the center (Fig. 1). Sleeves were dipped in a 50:50 mixture of blended whole eggs and corn oil (M.C. Vargas, pers. comm.) to which we added either 25 mg/bait THCL or 9 to 12 mg/bait of DuPont dye. The baits were shaken in a plastic bag containing fish meal until evenly coated and then removed and stored at 4 C to avoid mold growth.

Two types of bait field trials were undertaken. The first test, conducted on Blackmans, consisted of placing several baits at numerous widely spaced central locations with the expectation that visits to the site by different animals would result in a high proportion of the population locating and consuming baits (Linhart et al., 1993). The second test, conducted on the Langfords and Bodkins study sites, involved hand placement of single baits uniformly spaced along transect lines.

On Blackmans, 400 DuPont polymer baits containing both THCL and DuPont dye were distributed evenly in a grid pattern at 36 bait stations arranged at 150-m intervals on the 80-ha study area. The 150-m spacing between bait stations was based on a 0.51-ha home range size estimate for female mongooses (Pimental, 1955). Baits were distributed at a density of five baits per ha or about 11 baits at each of the 36 stations. Bait distribution began shortly after sunrise on 26 April 1991, and was completed by 1000 so as to maximize uptake by the diurnally active mongooses. Baits at 10 of the stations were checked at 1730 on the same day of placement to evaluate the removal rate.

For 2 days following bait placement, live traps were set at 20 m intervals along a dirt road that surrounded the inner 28 ha core area. The outlying 52 ha were not trapped and served as a buffer area to reduce the effects of mongoose movement into and out of the central core area. Captured mongooses were anesthetized, weighed, and had their sex determined. A 3 ml blood sample was taken from each by heart puncture with a 5 ml syringe and 21 ga needle; each animal then was euthanized. Abdominal, femoral bone marrow, lower mandibular, and tail fat were inspected visually for DuPont dye, either at the time of euthanasia or later on the day of capture in the laboratory.

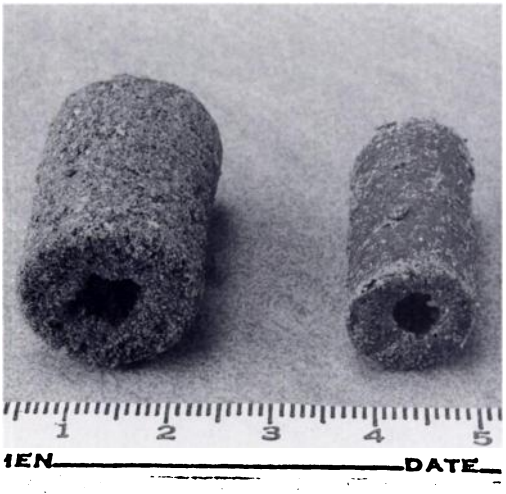

FIGURE 1. Polyurethane sleeve bait (left) and DuPont polymer bait (right) used for mongoose bait trials on Antigua, West Indies.

On the Langfords and Bodkins sites, 2,000 polyurethane baits and 2,000 DuPont fishmeal baits, respectively, (20 baits/ha) containing THCL and 400 identical baits per site (four baits/ha) containing DuPont dye were individually hand-placed along transect lines. Baits were placed at 10 m intervals along 24 transect lines spaced 40 m apart. Baits were placed along the transects in a sequence of five baits containing THCL, then one bait with DuPont dye, such that five times as many THCL baits as DuPont dye baits were distributed within the study site. This procedure was used to determine the percentage of trapped mongooses that were marked with THCL, DuPont dye, or both, and thereby to determine the percentage of animals marked at the different bait densities. A similar procedure was used at both the Langfords and Bodkins test areas, except that polyurethane baits were placed at Langfords and DuPont polymer baits were placed at Bodkins. On both Langfords and Bodkins, mongooses were live-trapped to determine population density and the percentage marked. Density estimates were made using a trapping-web and transect line methodology (Anderson et al., 1983). The day after bait distribution, 82 live traps were set on the center 36-ha core area of both study sites. Each trapping-web consisted of eight lines of 10 traps with the first trap in each line set 15 m from

FIGURE 2. Mongoose (*Herpestes javanicus*) at a 30 × 30 cm tracking tile with bait on Antigua, West Indies (photographed with an automatic camera).

the center, and the remaining traps set at 30-m intervals. Two additional traps were set at the center of the web. The surrounding 64 ha served as a buffer area. Mongooses captured during the first 3 days of trapping were evaluated for biomarkers as previously described. Trapping for the population estimates was conducted for a total of 8 days. The trap-web protocol required that all mongooses captured from the study area be removed. Therefore, those animals captured after day 3 of the bait trial also were anesthetized and euthanized but not examined for biomarkers.

We modified a tracking tile technique and used it on the Bodkins study site to evaluate bait consumption rates and to determine the percentage of baits consumed by mongooses versus non-target species (Lord et al., 1970; Linhart et al., 1993). The tiles consisted of 30 × 30 cm fiberglass sheets partially covered with a mixture of black printer's ink (Superior Printing Inks, New York, USA) and mineral oil (150 g ink/liter oil). The mixture was applied to the outer 7.5 cm of each tile, and a bait was placed on a 225 × 225 mm sheet of white paper in the center of the tile (Fig. 2). Tiles were placed at the first bait location at each of the 24 transects. The habitat type for these 24 locations was described as either pasture, brushy pasture, or brush, depending on the amount of cover present. Animal tracks present on stations and the disposition of the DuPont polymer baits were recorded at the end of the day on which baits were placed. Photographs of mongooses were obtained (Fig. 2) at two tracking stations by use of Trailmaster® automatic cameras (Goodson and Associates, Shawnee, Kansas). Statistical comparisons for significance of results were evaluated using the G-test for goodness of fit (Sokal and Rohlf, 1981).

RESULTS

Sections of the lower mandible from nine of the 11 animals that ate THCL and DuPont dye-treated polymer baits had the characteristic fluorescence of THCL up through day 12. One mongoose was negative for THCL, and one mongoose escaped on day 5 and was not available for examination. All 12 animals administered THCL via a feeding tube had THCL fluorescence through day 12 when the last group was euthanized and the biomarker phase of the study concluded. Animals dosed with THCL via feeding tube were more strongly marked than those fed the DuPont bait containing THCL.

DuPont dye generally failed to mark mongooses past day 1 of the study, regardless of whether given by bait or by feeding tube. Mandibular bone marrow fat (100%) and ventral tail fat (67%) proved to be the only locations marked by dye on day 1 by both routes of administration. Only one of 12 animals given bait and one of 12 animals dosed via feeding tube were positive on day 3. The tail fat of both were marked by the dye. Mandibular and abdominal fat were negative for all animals examined past day 1. The abdominal and femur marrow fat of test animals was only lightly marked or not at all.

We checked baits at 10 of the 36 bait stations on the Blackmans study area. At 8 hr post-bait placement, 80% of the 400 baits were gone, 6% were partially eaten, and 14% were intact. All 11 baits had been removed at six of the 10 bait stations. After 24 hr, 89% of the baits were gone and 90% were missing after 48 hr. The baits uneaten after 24 hr were located at stations where remote cameras had been placed; we believe that these devices may have fright-

ened mongooses and kept them from eating baits.

Fifty-five mongooses (32 males, 23 females) were captured in 132 trap-days (number of traps × days of operation) during the 2-day period following bait distribution. Mean (±SD) weight of 32 males was 664 (±67) g (range 500 to 770 g). For females the mean (±SD) weight was 450 (±83) g (range, 390 to 560 g). Among mandibles sectioned for THCL analysis, 38 (69%) of 55 were marked (Fig. 3). Males (81%) were marked at a greater frequency ($P \leq 0.01$) than females (52%). Based on gross examination of fat deposits for DuPont dye, 37 (67%) of 55 mongooses were marked. There was no significant difference ($P > 0.1$) between the numbers of marked males (24 of 32, 75%) and females (12 of 23, 52%). Mandibular bone marrow fat (63% of all animals) was the most consistent location of dye deposition. Dye was grossly visible 51% and 47% of the time, respectively, in the tail and abdominal fat. Mandibular bone marrow fat was marked in nine mongooses (eight males, one female) that were negative for dye in the tail and abdominal fat. Tail fat was marked in two female mongooses that were negative for dye in the mandibular and abdominal fat.

Results of bait distribution on the Langfords and Bodkins study areas were similar. After 3 days of trapping following bait placement, we caught 45 mongooses (23 males, 22 females) in 162 trap-days on Langfords, and 66 mongooses (32 males, 28 females) in 158 trap-days on Bodkins. Mean (±SD) animal weights on Langfords and Bodkins for males were 644 (±218) g (range, 250 to 920 g) and 588 (±197) g (range, 270 to 940 g), respectively. For females, they were 416 (±82) g (range, 220 to 560 g), for Langfords and 392 (±95) g (range, 200 to 530 g) for Bodkins.

Of the mandibles sectioned for THCL analysis, fluorescence was detected in 41 (91%) of 45 of the mongooses from Langfords and 60 (91%) of 66 from Bodkins (Fig. 3). Tetracycline was detected in significantly ($P \leq 0.01$) more females (22 of 22, 100%) than males (19 of 23, 83%) on Langfords, but no sex difference was found on Bodkins (males, 32 of 35, 91%; females, 28 of 31, 90%). Twenty-seven (60%) of the 45 mongooses sampled on Langfords were marked by DuPont dye; on Bodkins, 27 (41%) of 66 had dye present in fatty tissue. For both study areas, there were no significant differences between the percentages of males and females marked by dye. Tail fat was the most consistent location of dye deposition (26 of 45, 58%) for Langfords; (24 of 66, 33%) for Bodkins.

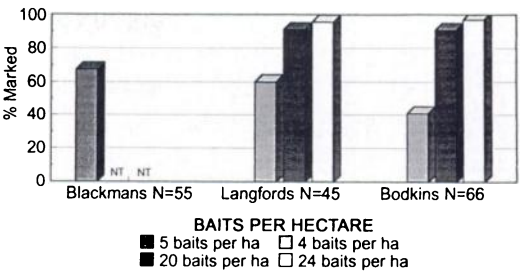

FIGURE 3. Percent of mongooses found marked at different bait densities on three study areas in Antigua, West Indies. DuPont baits were tested at Blackmans and Bodkins, polyurethane baits were tested at Langfords. Sample sizes are shown in parentheses. NT = Not Tested.

At the Langfords site, where polyurethane baits were used, a bait acceptance rate was calculated for a bait density of 24 baits per ha by combining the THCL and DuPont Oil Blue A® dye results. Forty-three (96%) of 45 mongooses sampled were marked by either biomarker at this maximum bait density (Fig. 3). Twenty-three (51%) of 45 of the mongooses sampled were marked by both THCL and DuPont Oil Blue A® dye. During a 10-day trapping period following bait distribution, 75 mongooses were captured in 636 trap days. Dates and locations of captures were used to compute a mean (±SE) population density estimate of 5.84 (±1.04) mongooses per ha using the modified trapping web procedure. At this population density, there were approximately 0.68 baits available per mongoose when baits were distributed

at a density of four per ha. When baits were distributed at densities of 20 and 24 baits per ha, 3.42 and 4.10 baits were available per mongoose, respectively.

For Bodkins, 105 mongooses were trapped in 630 trap days during the 10 day post-baiting period. A mean (±SE) mongoose population density estimate of 5.75 (±1.04) mongooses per ha resulted in about 0.4 baits per mongoose at a baiting density of four baits per ha. When baits were distributed at 20 and 24 baits per ha, 3.48 and 4.17 baits were available per mongoose, respectively.

Similarly, at the Bodkins site where DuPont polymer baits were used, a bait acceptance rate was calculated for a bait density of 24 baits per ha by combining the THCL and DuPont dye results. Sixty-four (97%) of 66 of the mongooses sampled were marked at this bait density (Fig. 3). Twenty (30%) of 66 mongooses sampled were marked by both THCL and DuPont dye. Thus mongoose bait uptake was similar for both the Langfords and Bodkins area, regardless of which bait type was used.

Based on the tracking tiles at the Bodkins site, 15 (63%) of the 24 baits were gone within 3 hr of their distribution. All 14 baits at stations in brush and brushy pasture habitats were taken, whereas only one of 10 baits located in open pasture habitat was removed. Mongoose tracks were present on all tracking tiles where baits had been removed within 3 hr.

DISCUSSION

We demonstrated that it was feasible to deliver oral biologics to free-ranging mongooses. We found that when baits were distributed at a density of 24 bait/ha, the baiting system would have potentially delivered an oral vaccine, toxin, or contraceptive to 96 to 97% of the mongoose population. This conclusion was based on the percentage of mongooses that were positive for at least one of the two biological markers. However, we recognize that the addition of a vaccine container might alter the acceptance rate. Also, our tests were conducted on relatively small study areas and additional field tests on much larger study areas are needed.

Tetracycline HCL was observed in marked captive mongooses for the duration of a 12-day-long biomarker test. It also proved satisfactory during each of the three subsequent field trials. DuPont Oil Blue A® dye failed to consistently mark captive mongooses for longer than 24 hr. Intuitively, the efficacy or retention of this marker should depend upon the amount of fat available for dye deposition. Mongooses used in April 1991 for our biomarker test were captured at the end of the dry season when the food supply was limited. Consequently, there was very little abdominal or tail fat visible in these animals. Mean (±SD) weights for six males and 17 females were 623 (±38) g and 423 (±49) g, respectively. In contrast, 17 males and 13 females captured earlier at the same location in February 1991, had means of 710 (±102) g and 465 (±46.7) g, respectively. Thus there was a significant ($P < 0.01$) decrease in fat reserves between February and April. However, the difference also could have reflected a change in age distribution within the local population. The effects of capture and diet-induced stress on mean weights of captive mongooses were not likely to have been a factor because animals used in the biomarker trial were weighed within 24 hr of capture.

Both THCL and DuPont dye were incorporated into the same bait for the central point bait station test on Blackmans. This procedure provided a means of comparing their relative efficacies which proved to be almost identical (69% positive for THCL; 67% marked by DuPont dye). Mean (±SD) mongoose weights on Blackmans were the highest recorded (males, 664 ± 67 g; females, 450 ± 83 g) for any of the study areas. The strong agreement between biomarker results, coupled with the high average weights, supported the contention that DuPont dye deposition was directly related to the amount of fat pres-

ent in target animals. However, since multiple baits were distributed at each station for this trial, an alternative and probable explanation is that the marked animals had consumed more than one bait.

Placement of multiple baits at central point stations required fewer personnel-hours than did placement of single baits along transects. However, the spacing of the bait stations at 150 m intervals appeared to have caused a home range size-related bias towards males that was not apparent when baits were distributed along transects. Mongoose home range sizes in Puerto Rico were estimated at 0.51 ha for females and 1.23 ha for males (Pimental, 1955). As indicated by G-test analysis, significantly ($P < 0.01$) more males (81%) were positive for THCL than females (52%) when baits were distributed at stations. In comparison, significantly ($P < 0.01$) more females (100%) were positive for THCL than males (82%) on Langfords, and no significant difference was evident between males (91%) and females (90%) on Bodkins where single baits were distributed along transects. Peak breeding occurs in mid-June so mongoose movement patterns during our study should not have been affected by breeding or denning behavior. Social factors such as competition for food or scent marking of bait stations by dominant animals also may have influenced consumption rates.

Although our results were promising, the practicality and efficacy of the bait delivery system needs further study. Obviously, the number of baits distributed should exceed the number of target animals in the study area. The population density estimates for Langfords and Bodkins were 5.84 and 5.75 mongooses per ha, respectively. When baits were distributed at the lower density of four baits per ha, there were only about 0.7 baits available per mongoose as compared to approximately four baits available per mongoose at the maximum baiting density of 24 baits per ha. Since it is unlikely that 96 to 97% of mongoose populations need to be vaccinated to control rabies, the distribution of excess baits would result in unnecessary labor and expense. Further study is therefore needed to determine optimal baiting densities under the different conditions that prevail on islands where the disease is a problem. Because mongooses prefer dense brushy areas, aerial bait distribution should increase access to mongooses concentrated in such habitat. Thus, study of bait placement by air should receive a high research priority.

Seasonal influences on habitat, food availability, and bait uptake need further investigation. For example, the dry season in Antigua results in a dramatic reduction in grassy ground cover and the associated insects that comprise the bulk of a mongoose's diet. Not only would bait distribution during this period target mongooses at a time when their food supply was limited, but reduced ground vegetation would also tend to concentrate mongooses in areas of suitable cover and thus facilitate bait discovery.

In an earlier study by Linhart et al. (1993), and in the field trials described herein we failed to show any discernible preference among baits. Opportunistic feeding behavior by mongooses may eliminate the need for custom-formulated baits to meet local mongoose food or bait preferences. Nonetheless, it may be necessary to modify baits to meet the specific requirements of a vaccine, toxin, or contraceptive. For example, vaccines must be placed in protective containers to prevent loss of potency from contact with the bait material whereas toxins can usually be mixed directly into the bait material with no loss of biological activity.

Because the mongoose is an introduced species in the Caribbean region and Hawaii, population reduction using toxic baits may be a viable management approach. This is particularly true where mongooses are adversely impacting native or endangered wildlife and where the need for control is limited to small geographic areas. Alternatively, the use of an inexpensive toxicant to reduce population density fol-

lowed by the distribution of a reduced number of more costly vaccine baits may be a viable and acceptable control strategy. Finally, as efforts to control the disease in dogs through parenteral vaccination becomes more effective, the mongoose will become even more important as a rabies reservoir and carrier. The development of techniques to deliver an oral rabies vaccine to free-ranging mongooses should provide a useful method for the control and management of the disease in this species.

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