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Acceptance of candidate baits by domestic dogs for delivery of oral rabies vaccines

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ABSTRACT

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Protocols for evaluating oral rabies vaccine baits for domestic dogs were field tested in central Mexico, after which dog-food manufacturers and suppliers to the pet-food industry were advised as to potential ingredients for use in prototype dog baits. Bait-preference trials in which confined dogs were used were then undertaken, followed by field tests of free-ranging farmer-owned dogs in three towns in the Nile River Delta region of Egypt. Both confined and free-ranging dogs showed strong preferences for certain baits or bait coatings (poultry, beef tallow, cheese, egg and a proprietary product). Fish-meal polymer baits, widely used for wildlife species, were less preferred. In Egypt, a commercial dog-food-meal bait coated with beef tallow and dry cheese was consumed at a rate approaching that of a chicken-head bait.

The percentage baits that were actually eaten after they had been offered to dogs, ranged from 71–96 % for household dogs tested in Mexico, 65–91 % for confined dogs (beagles and mixed breeds) tested in the United States, and 32–88 % for farmer-owned dogs tested in Egypt.

Keywords: Baits, domestic dogs, oral vaccine, rabies

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INTRODUCTION

Effective control of dog rabies in many developing countries is hindered by the presence of large numbers of unvaccinated free-ranging dogs [World Health Organization (WHO) 1988; Wandeler, Matter, Kappeler & Budde 1993]. The success of oral rabies immunization of red foxes (*Vulpes vulpes*) in Europe (Aubert, Masson, Artois & Barrat 1994) and Canada (MacInnes, Nunan, Lawson, Campbell, Bachmann, Tinline, Pond, Johnston, Calder & Pedde 1993), and the potential for immunizing raccoons (*Procyon lotor*) in the United States (Rupprecht, Hanlon, Niezgoda, Buchanan, Diehl & Koprowski 1993), has led the WHO to recommend use of vaccine-laden baits for dogs in lieu of or in conjunction with parenteral vaccination (WHO 1991).

An important requirement for oral vaccination is a readily ingested bait for delivering the vaccine. Several candidate baits, originally developed for vaccine delivery to red foxes or raccoons, have been tested for dogs, with the hope that they would be equally acceptable. However, dog acceptance of such baits has been quite variable. Baits evaluated for dogs have included a plastic film-covered polyurethane sponge bait coated with chicken-flavoured wax and tallow, and were used in conjunction with a liquid attractant to enhance discovery (tested in Zimbabwe and Tunisia: Perry, Brooks, Foggin, Bleakley, Johnston & Hill 1988; Kharmachi, Haddad & Matter 1992); a cooked and deep-fried corn-meal bait (tested in Zimbabwe: Baer, Brooks & Foggin 1989); chicken heads (tested in Tunisia, Turkey and Nepal: Kharmachi *et al.* 1992; WHO 1993, 1994); a sausage bait of donkey meat and cooked rice (tested in Tunisia: Kharmachi *et al.* 1992); the German "Tübingen" fox bait and a local sausage termed the "Köfte" bait (tested in Turkey: WHO 1991, 1994); the DuPont de Nemours (Wilmington, DE, USA) polymer fish-meal bait (tested in Tunisia and Mexico: Frontini, Fishbein, Ramos, Collins, Torres, Huerta, Rodriguez, Belotto, Dobbins, Linhart & Baer 1992; Kharmachi *et al.* 1992); a Canadian blister-pack bait (Bachmann, Bramwell, Fraser, Gilmore, Johnston, Lawson, MacInnes, Matejka, Miles, Pedde & Voigt 1990) consisted of tallow, waxes, and chicken or beef flavours (tested in Mexico and Nepal: Frontini *et al.* 1992; WHO 1994); and a cylindrical polyurethane sponge bait coated with a corn-egg-and-milk-batter mixture, and deep-fried in corn or fish oil (tested in Mexico: Frontini *et al.* 1992). A commercially available dog biscuit, or a cylindrical dog-biscuit-meal prototype bait formulated with an edible polymer and initially made for us by DuPont de Nemours in 1991, has been used either as a reference or control-food item (tested in Mexico: WHO 1991; Frontini *et al.* 1992), or as a prototype bait (tested in the United States: Linhart & Wlodkowski, unpublished data, Nepal, WHO 1994). Two proprietary baits fabricated by Virbac Laboratories (Carros, France) have been tested in Tunisian dogs (WHO 1994).

The above-mentioned baits have been tested randomly or sequentially by going door-to-door and presenting either single or paired baits (two different types) to individual household dogs. Dog ingestive behaviour was recorded and bait preference generally expressed as the percentage of each type eaten. Less frequently, unattended baits were left for free-ranging dogs at tracking stations (sites having sifted or raked soil) and placed at refuse dumps or slaughter houses. Stations were later checked for animal tracks and other signs, and the results expressed as the percentage of stations visited by animals and the number of each bait type taken by target (dog) and nontarget species. These studies have recently been summarized (Linhart 1993; WHO 1994; Linhart, Kappeler & Windberg, in press).

The bait studies described in this paper were conducted to:

- evaluate different field-test protocols by the use of dogs in rural central Mexico;
- systematically formulate candidate baits specifically for dogs;
- determine bait preferences of confined beagles and mixed-breed dogs in the United States; and
- assess bait preferences and acceptance by mixed-breed dogs owned by farmers in the Nile River Delta region of Egypt.

MATERIALS AND METHODS

Bait-test protocols—Mexico (1990)

This phase of the research focused on an evaluation of different protocols for determining bait preferences and acceptance. Four investigators (Linhart, Flores Collins, Balderas Torres and Baer) undertook field tests in January 1990 and again in May–June 1990 in three towns (La Soledad Morelos, Tezonteopan de Bonilla and Teacalco) and nearby areas in the state of Puebla, located in the central highlands of Mexico. The test areas and associated human and canid demographics have previously been described (Fishbein, Frontini, Dobbins, Collins, Huerta, Rodriguez, Woo-Ming, Ramos, Belotto, Torres, Yenne, Linhart & Baer 1992; Frontini *et al.* 1992). The field trials were undertaken to compare four different bait-test protocols.

Street dogs—single-bait choice

Each two-member team was provided with a supply of four different bait types (a–d described below) and assigned to designated test areas within the towns of Soledad and Bonilla. Teams issued with maps of the town were instructed to walk the streets systematically and to present a single bait to each free-ranging dog they encountered. The type of bait and order of presentation were randomly determined so that each bait was offered to the same number of dogs. One team member offered the bait and timed the events, while the second recorded data. Each dog was given 3 min to investigate and consume the bait. An attempt was made to test this protocol on 100 dogs (25 dogs per bait type).

Household dogs—single-bait choice

This protocol was evaluated in the town of Teacalco by use of the same procedure as described in protocol I, except that the teams went from house to house and, upon permission from household members, tested dogs that were physically located within the house or courtyard. This procedure was similar to that described by Frontini *et al.* (1992). Dogs were offered baits, and data were recorded as in protocol I above. Differences among proportions of each bait type consumed were analyzed by means of Pearson's chi-square test (Snedecor & Cochran 1980).

Household dogs—two-bait choice

This protocol involved offering household dogs two different bait types simultaneously, i.e. the two-choice preference test normally used by the pet-food industry and animal psychologists to determine the food preferences of animals. Each test was conducted by placing the two candidate baits on the ground approximately 25 cm apart in front of the dog and, along with other data, recording which of the two baits was taken or eaten first (i.e. preference). Dogs were offered a choice of all six possible bait-type combinations (ab, ac, ad, bc, bd, cd) in random sequence for a total of 168 trials (28 dogs/bait combination). No more than two dogs per household were tested.

Groups of street dogs

A two-person team approached groups of dogs (two or more animals) at a public market, a refuse dump and a municipal slaughter house and attempted to feed a single bait (bait-b type only) to each dog within the group. The number of offered baits was limited to one bait for each dog in the group. Baits were offered by tossing or dropping a bait in front of one dog, and when that individual was occupied, offering additional baits, one at a time, to other group members. The extent of competition for baits among dogs within each group was ascertained by noting aggressive or submissive behaviours, and which dogs ate single or multiple baits or were unable to obtain one.

Bait types tested—Mexico (1990)

The test protocols described above were used, and the four different bait types compared as follows:

- Commercial dog biscuits were found to be highly acceptable to dogs in Mexico (Frontini *et al.* 1992). A bait (a) was therefore prepared by drilling 2-mm-deep holes into one-half of a large-size (1,5 x 5,0 x 5,5 cm) commercial dog biscuit (Purina Mills). The holes were lined with melted paraffin wax and then filled with 1 mℓ of water coloured with food dye (this bait provided by Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, 30333 Georgia, USA).
- For bait b, a cylindrical open-celled polyurethane sleeve (1,5 cm x 5,5 cm) was saturated with a milk-egg-and-corn-based batter, (Linhart, Blom, Dasch, Roberts, Engeman, Esposito, Shaddock & Baer 1991; Frontini *et al.* 1992). The bait was then deep-fried in paraffin oil and air-dried until hard. A paraffin-wax ampule containing 1 mℓ of water coloured with food dye (placebo vaccine) was inserted into each bait.
- Bait c was the same as bait b above, but approximately one-half its length (1,5 cm x 3,0 cm).
- For bait d, a hole was cored in a length of commercially available beef sausage (hot dog) to accept a water-filled (1,5 mℓ) paraffin-wax ampule. Bait was air-dried and hardened for 4 d at ambient temperature, resulting in a bait 1,5 cm x 4,5 cm in size.

Bait trials with confined dogs—USA (1994)

After the 1990 bait-test protocols in Mexico had been analysed, dog-food manufacturers and suppliers to that industry were contacted for suggestions as to ingredients and enhancers that might be incorporated into vaccine baits. After extensive inquiry and formulation, eight bait types were selected and 14 topical flavour coatings (Fig. 1 and Table 1) for evaluation. Industry recommendations as to concentrations were followed to formulate nearly all flavour enhancers (both liquid and dry); i.e. about 0,5–5% of total food (i.e. bait) mass. It was found that about 3% (by mass) topically applied liquid products would thoroughly coat the exterior surface of the standard dog-food bait, and that a maximum of about 3% dry powder or meal products would adhere to the liquid coatings. The 3% concentration rate was therefore used for all candidate flavour enhancers unless otherwise stated.

The standard dog-food bait as fabricated for the study by DuPont de Nemours was coated and used to compare candidate flavours. It consisted of an extruded cylinder (2 cm x 5 cm) made of 88% Purina Mills Canine Diet Number 5006 (meal), 10% ethylene vinyl acetate (EVA) (ELVAX 150®, a DuPont de Nemours product), and 2–3% vegetable oil as an extruder lubricant (bait A, Table 1). The bait contained a longitudinal hole (1 cm in diameter) for insertion of a vaccine container. This relatively odourless bait was used only once as a matrix bait material or base substrate to be surface-coated with various combinations of liquid, powdered, or granular attractants, termed "enhancers" by the pet-food industry. This bait was used to sequentially test the 14 flavour coatings on both confined and free-ranging dogs.

Candidate baits and bait coatings were tested in the spring of 1994 on 20 adult beagles confined at the U.S. Public Health Services' Centers for Disease Control and Prevention (CDC) animal containment facility near Atlanta, Georgia, USA. Twenty adult mixed-breed dogs confined at the College of Veterinary Medicine, The University of Georgia (UGA), Athens, Georgia, USA, were subsequently tested according to the same protocol and with nearly all the identical baits and coatings. Water was available *ad libitum*, and the customary ration of dog food was provided after each daily test. Tests were conducted from 08:30–10:00 with the use of a two-choice bait test that involved presenting each dog with a plexiglass tray holding two disposable, plastic food cups

TABLE 1 Baits and bait coatings offered to confined dogs (USA) and farmer-owned dogs (Egypt)

Bait designation	Bait/bait-coating composition
A-	~88% dog-food meal (Purina Mills Canine Diet #5006)[a] 10% EVA (polymer), ~3 % vegetable oil[b]
B-	Fish meal, fish oil, EVA (polymer), proprietary formulation[b]
C-	45% Plastisol[c] 20% Plastisol hardener[c], 30% ALPO beef-flavored dog-food meal[d], 5% poultry oil
D-	Proprietary formulation[e]
E-	Polyethylene capsule[f] containing 3,5 mℓ mixture of rhodamine B dye and H_2O (100 mg/250 mℓ solution). Capsule dipped in mixture of 97,5% melted adhesive[g] and 2,5% poultry oil, then rolled in dried poultry biodigest[h]
F-	Beef sausage, uncooked
G-	Chicken head
H-	45% beef-flavored ALPO dog-food meal, 30% paraffin wax, 20% "trap" wax, 5% poultry oil
Bait/coating designation	**Bait coatings**
A1	3–4% Menhaden Fish Oil overcoated with 3% fish meal
A2	3% rendered beef tallow overcoated with 3% Cheese Plus[i]
A3	3% rendered beef tallow overcoated with 2% liver meal
A4	3% rendered beef tallow overcoated with 3% Made For Dogs (MFD)®[j]
A5	3% unrefined corn oil containing 3% sweet-corn essence oil[k]
A6	3% poultry oil overcoated with 3% dried poultry biodigest
A7	3% liquid lamb digest[h] coated with 3% ground Purina O.N.E. Lamb and Rice Dog Food Meal
A8	3% liquid beef digest overcoated with 3% meat/bone-scrap meal
A9	3% rendered beef tallow overcoated with 1 % instant onion
Bait/coating designation	**Bait/bait-coating composition**
A10	3% liquid Real Egg Product[l] overcoated with 3% inedible dried whole egg
A11	3% mixture of 50% peanut butter and 50% peanut oil overcoated with 3% corn starch
A12	Local bread dough coated on bait A and baked
C13	3% dried poultry biodigest
H14	1 % dried poultry biodigest

a Purina Mills, St.Louis, Missouri, USA
b DuPont de Nemours, Orange, Texas, USA
c MF Manufacturing Co., Fort Worth, Texas, USA
d ALPO Pet Foods, Lehigh Valley, Pennsylvania, USA
e Bio-Serv Inc., Frenchtown, New Jersey, USA
f Kafco International Ltd., Chicago, Illinois, USA
g J.T. Eaton and Co., Twinsburo, Ohio, USA
h Bioproducts, Louisville, Kentucky, USA
i International Ingredients, St. Louis, Missouri, USA
j Mondovi Foods Corp., Mondovi, Wisconsin, US
k M&M Furs, Bridgewater, South Dakota, USA
l Worthington Foods Inc., Worthington, Ohio, USA

(3 cm apart), each of which contained a different bait type. Dogs were offered commercial dog biscuits in bait trays for 3 d prior to starting bait trials, to accustom them to the test procedure. For actual trials, all 20 dogs received identical two bait types or coatings on a given day. Each dog was observed for a 3-min period during the trial, and the order in which both bait types were picked up and eaten was recorded. Trays were then removed from shelters and cleaned, containers replaced, kennels cleaned, and dogs fed. The percentage of each bait or coating type that was eaten first was calculated for each test day. The bait type that was more frequently eaten (> 50%) on a given day was advanced to the next day's test and compared with a new type of bait or bait coating. The bait eaten less often (< 50%) was dropped from further testing. These two-choice "elimination"-type preference trials were continued until all candidate baits and coatings had been exposed at least once to all 20 dogs. Candidate baits that survived phase 1 were paired and tested again (phases 2–4) until the final two "best" baits were compared and the surviving bait then compared with the untreated control bait.

Bait trials with Egyptian dogs

Field trials were conducted in Egypt in June 1994 in three towns (Mashûl el Sûq, Salamant and Kafr Ibrash) in the governorate of Sharkia, located about 50–75 km north of Cairo in an area of intensive but diverse irrigated agriculture. Nearly all test dogs were owned by farmers living either on the periphery of larger towns or in adjacent communities. Criteria used to select baits and bait coatings for the field trial included results from the previous tests on confined dogs; baits previously used by other investigators for several species, including dogs (i.e. DuPont polymer fish-meal bait); and commercial bait types that had possible potential for large-scale production. Lastly, the number of test baits was limited by the time available for field trials. The research team initially elected to test six different bait types and coatings (baits A,B,

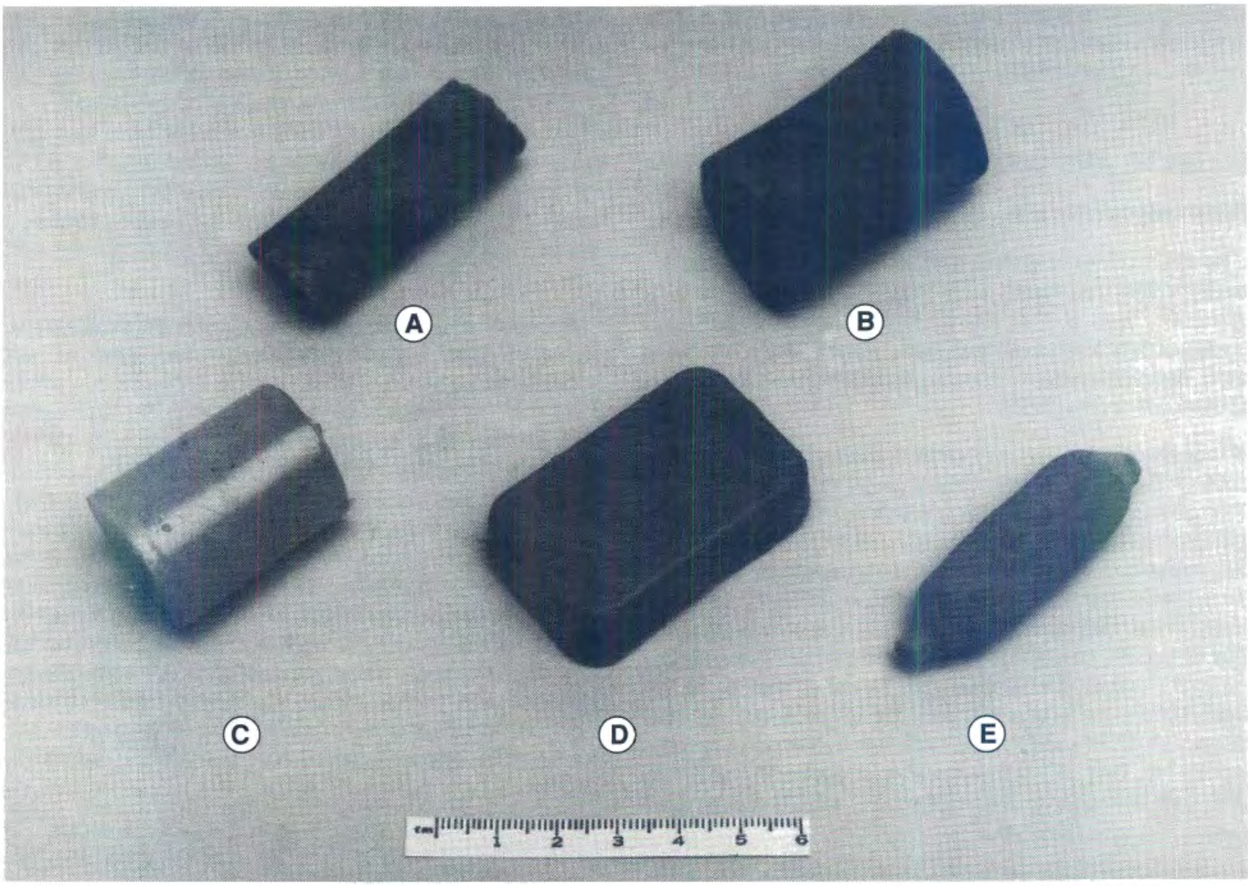

FIG. 1 Several bait types tested in confined (USA) and free-ranging (Egypt) dogs, 1994

A = dog-food meal and polymer
B = fish meal, fish oil, and polymer
C = Plastisol and beef-flavoured dog-food meal
D = proprietary formulation from Bio-Serv, Inc.
E = polyethylene vaccine container coated with poultry-flavoured adhesive (see Table 1 for details)

D, A2, A4, A6, Table 1), but because field work progressed more rapidly than anticipated, four additional baits (F, G, A10, A12, Table 1) were subsequently tested, three of which were obtained and prepared locally.

It was most effective to conduct bait trials from about 17:00–24:00 when farmers and their dogs were at home after they had completed their daily work in the fields. The local veterinarian or his assistant preceded the bait-evaluation team along streets or alleyways, contacted dog owners or family members, briefly explained the project, and requested permission to use their dogs as test animals. Because the Egyptian dogs were found to be more wary and easily frightened than the Mexican dogs tested in 1990, it was difficult to approach them consistently and position two baits equally distant so as to ensure an equal opportunity for selection and consumption (two-choice test). Therefore, of necessity, a single-choice bait test was conducted by presenting a single bait type to each test dog, (i.e. protocol II, Mexican test). Each dog was given 3 min to consume the bait or, if feeding commenced toward the end of the 3-min period, the dog was given an additional 2 min (total of 5 min) to finish ingestion. Bait types or coatings were presented sequentially as individual dogs were encountered (e.g. the first dog was offered bait A, the second, bait B, ... the sixth, bait A6). This sequence of bait presentation was repeated until a sample of about 35 individual dog trials per bait type or coating had beem obtained. The same procedure was used for testing the four supplemental baits.

It was decided not to drive or walk through streets or alleyways and present baits to free-ranging dogs unaccompanied by owners, because it was not known what the community response would be to this procedure. Local authorities stated that nearly all dogs were owned and therefore could be offered baits at or near their owners' dwellings.

By use of the above procedure, 30–40 dogs were tested and their responses recorded each evening during the 10-d test period. Differences between proportions of each bait type consumed were analysed by means of Pearson's chi-square test (Snedecor & Cochran 1980).

RESULTS AND DISCUSSION

Bait-test protocols—Mexico (1990)

The mean time required to conduct a single-bait preference trial by means of protocol I (street dogs—single bait) was 9 min, 30 s (n = 103); for protocol II (household dogs—single bait) 7 min, 0 s (n = 100); and for protocol III (household dogs—two baits) 9 min, 30 s (n = 165).

It is believed the behaviour of the street-dog subpopulation that was offered baits (protocol I) was indicative of the degree to which such dogs could be vaccinated orally. In this regard, it was observed that household dogs were seldom restrained or confined and therefore sometimes became "street" dogs by simply wandering out of the house or courtyard into the adjacent street. Of 163 dogs seen on the streets, 132 (81%) were approached. Thirty of these (23%) fled before bait was offered. Of the remaining 102 dogs, 83 (81%) ate the bait offered, one partially consumed its bait, 15 dogs refused the offering (15 %), and the remaining four dogs fled with the bait and could not be observed.

Mean bait-chewing times varied in accordance with bait types and appeared to be related to bait size and possibly bait friability, i.e. 146 s for bait a (largest size), 78 s for bait b (intermediate size), 44 s for bait c (small size), and 51 s for bait d (small size). Except in the case of bait a, these means fell within the ranges of the biscuit and bait types consumed as reported by Frontini *et al.* (1992). No obvious relationship between size of dog and the time required to consume baits was observed. Time required for bait mastication showed great variation among individual dogs. For example, for dogs tested under protocol II (n = 100), mastication times ranged from 14–310 s for bait a, 4–320 s for bait b, 4–180 s for bait c, and 2–199 s for bait d. Pieces of bait falling to the ground during mastication were observed to occur 77% of the time and did not vary greatly among bait types.

Regardless of type, single baits offered to household dogs (protocol II) were completely eaten 89% of the time (range 84–96%), partial consumption was infrequent (4%), and uneaten baits were recorded for only 7% of total trials. Intact ampules and pieces of ampules or chewed portions of them were recorded at 3% and 14%, respectively. For baits b, c and d, the percentage of each where spillage of placebo vaccine was observed, occurred in 32%, 25%, and 70% of tests, respectively. These results, at least for baits b and c, appeared more conducive for vaccine delivery than those reported by Frontini *et al.* (1992).

No spillage of bait a was observed, because the placebo vaccine had been absorbed into the biscuit, the paraffin-lined holes having failed to contain the liquid. There were no differences (χ^2 = 2,481, d.f. = 3, P = 0,479) in the proportions of each of the four bait types consumed (84% for bait a, 96% for bait b, 91 % for bait c, and 84% for bait d). Therefore, while protocol-II data indicated excellent bait acceptance, it did not demonstrate any preferences between bait types.

The two-choice household-dog bait test (protocol III, Table 2) revealed a somewhat lower level of bait acceptance (mean = 79%; range = 71–85%), while the range in levels of acceptance among bait types was similar to protocol II (i.e. 14% versus 12%). Analysis of the data (Table 2) generated by comparing all six possible bait combinations in the two-choice test (a versus b, c, d; b versus c and d; c versus d) revealed that when offered a choice, dogs selected some baits first, up to twice as often as they did others (c versus d, Table 2). However, only the c-versus-d comparison was marginally significant (P = 0,10). These data suggest that the two-choice protocol was preferable to the single-bait test (protocol II), but that it was still relatively insensitive at the sample sizes obtained (i.e. n = 20–27, Table 2).

TABLE 2 Bait preference of Mexican dogs in a two-choice bait-preference test (protocol III)

Bait types compared	n	% baits eaten first (bait 1/bait 2)	χ^2 (1 d,f,)	P-value
ab	23	52/48	0,044	0,83
ac	27	59/41	0,926	0,34
ad	20	65/35	1,800	0,18
bc	26	38/62	1,800	0,18
bd	22	64/36	1,636	0,20
cd	24	67/33	2,667	0,10

The composition of the 15 groups of free-ranging mixed-breed dogs tested for bait acceptance (protocol IV) was eight pairs of dogs, one group of three dogs, three groups of four dogs, one group of five dogs, no groups of six dogs, and two groups of seven dogs (total of 50 dogs). The number of baits offered was limited to one bait for each dog in the group. Two of the groups (one group of four dogs and one of seven dogs) became frightened and fled when attempts were made to offer them baits. Of the remaining 13 groups, 33 baits (84,6%) were consumed, one was partially consumed, and five were rejected. Some degree of competition for baits in four of 13 dog groups (30,8%) was observed; however, none of the competing dogs in these groups was overtly aggressive or fought over baits. One or more baits were fed to 23 of the 39 dogs (60%) in the 13 groups; the remaining 16 dogs were not able to consume baits because these were taken by other pack members either more dominant or quicker to seek and obtain a bait.

As a basis for comparison, 81% (83 of 103) of the approachable, single, free-ranging street dogs ate offered baits completely (protocol I). These data indicated that some competition for baits occurred among that segment of the dog population that was found in groups. It is suggested that a few more baits could be used than the number of individual dogs within the groups to compensate for dominant behaviour and to increase the percentage of bait uptake by this subpopulation. Also, a lower percentage of the dog population was exposed to baits in highly urbanized areas (city parks and streets, municipal markets) because of traffic noise and a highly mobile human population that frightened dogs and made them less prone to pick up baits on busy sidewalks and walkways. The baiting of rural street-dog populations may therefore be somewhat less difficult than in urban situations, at least under the conditions encountered in central Mexico.

Bait trials with confined dogs—USA (1994)

The two-choice preference test demonstrated strong preferences among beagles and mixed-breed dogs for certain bait types and coatings (Table 3, Fig. 2). Several candidate baits were compared with the highly accepted dog-meal polymer bait coated with poultry byproducts (bait A6, Table 1). The latter bait was preferred over candidate baits made of Plastisol (bait C), fish-meal polymer (bait B), and the Bio-Serv bait (bait D). Among the 14 candidate bait coatings, dog responses varied from no preference (i.e., equal numbers of both bait types eaten first), to trials in which 17 of 18 dogs that ate baits, selected the same type first (phase 2, A6 versus A2, Table 3). Beagles preferred baits coated with poultry, Made For Dogs (MFD®), egg and cheese products, whereas fish-flavoured baits and those provided by Bio-Serv were the least preferred. A bait formulated of polyvinyl chloride (PVC), dog-food meal and poultry oil, ranked intermediate in preference. The observation that 18 of 20 beagles consistently ate baits whereas the remaining two individuals never ate any of the baits offered during the entire 3-week test period, was of interest.

The preferences of the mixed-breed dogs were similar to those of the beagles. These data were of interest because laboratory beagles were believed to be much more excitable and less discriminating than mixed-breed dogs. The mixed breeds also preferred baits and coatings of poultry, MFD®, egg and cheese, and showed less preference for fish-flavoured baits and those provided by Bio-Serv. One bait containing dog-food meal, paraffin wax, and poultry oil (bait H, Table 1) was tested on mixed-breed dogs but not on beagles. It was taken with the same frequency as were the dog-meal/poultry bait (A6) and the Plastisol bait (C, Fig. 2).

TABLE 3 Two-choice-preference elimination trials of bait coatings—beagles and mixed-breed dogs, USA, 1994

Bait/coating	% baits eaten first. Beagles (no. eaten/ no. offered)	% baits eaten first. Mixed breed (no. eaten/ no. offered)
Phase 1		
A2 cheese B-fish-meal polymer	– –	60 (12/20) 25 (5/20)
A2 cheese A3 liver	60 (12/20) 30 (6/20)	65 (13/20) 20 (4/20)
A4 MFD® A5 corn oil	60 (12/20) 30 (6/20)	60 (12/20) 20 (4/20)
A6 poultry A7 lamb	62,5 (12,5%/20) 20 (5/20)	50 (10/20) 40 (8/20)
A8 beef A9 onion	73,75 (14,75/20) 15 (3/20)	60 (12/20) 20 (4/20)
A10 egg A11 peanut butter	75 (15/20) 15 (3/20)	50 (10/20) 35 (7/20)
A1 fish B- fish-meal polymer	70 (14/20) 12,5 (2,5/20)	– –
Phase 2		
A4 MFD® A8 beef	85 (17/20) 5 (1/20)	45 (9/20) 45 (9/20)
A2 cheese A10 egg	35 (7/20) 55 (11/20)	– –
A1 fish A6 poultry	5 (1/20) 85 (17/20)	– –
A7 lamb A10 egg	– –	40 (8/20) 50 (10/20)
A6 poultry A2 cheese	– –	85 (17/20) 5 (1/20)
Phase 3		
A4 MFD® A10 egg	40 (8/20) 50 (10/20)	45 (9/20) 50 (10/20)
A2 cheese A6 poultry	45 (9/20) 45 (9/20)	– –
A6 poultry A8 beef	– –	47,5,(9,5/20) 45,(9/20)
Phase 4		
A6 poultry A10 egg	60,(12/20) 30,(6/20)	60 (12/20) 30 (6/20)
A6 poultry A- control	71,25 (14,25/20) 20 (4/20)	80 (16/20) 10 (2/20)

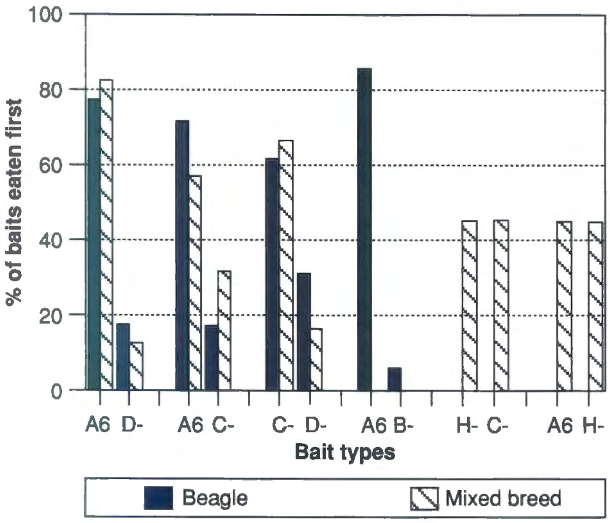

FIG. 2 Results of two-choice bait-preference trials with confined beagles and mixed-breed dogs, USA, 1994

A water-filled bait of 4-mℓ capacity, comprising a polyethylene capsule coated on the exterior with an adhesive and poultry oil, and then rolled in poultry biodigest (bait E, Table 1 and Fig. 1) was offered to 20 dogs (14 beagles, 6 mixed breeds) as previously described. Sixteen of 20 dogs chewed the capsule, seven capsules were swallowed (two of which had been punctured), and one dog refused the bait. Eighty percent (16/20) of these dogs ingested an unknown amount of placebo vaccine. The results of the above trial, as well as of a subsequent trial in which 70% (7/10) of thin-walled plastic pouches (Prodo-Pak Corporation, 77 Commerce Street, P.O. Box 363, Garfield, New Jersey 07026) coated with a poultry-flavoured wax were punctured, suggested that flavour-coated vaccine containers have considerable potential for enhancing vaccine delivery to dogs and perhaps to wildlife as well.

The two-choice bait test was found to be more sensitive to differences in preference than was the single-choice test. It appears to be a rapid means of determining preferences, provided the number of bait types to be compared is relatively small. In Mexico, it was found that the differences in the proportions of the four baits (a–d) eaten in the single-choice test, was only 84–96%, thereby indicating little difference in preference. However, when the same baits were tested in the two-choice trial, several baits were eaten first about twice as often as others (Table 2), thus providing a much better indication of bait preference. Using a 3-min test limit, one technician could sequentially test and observe 20 dogs in about an hour. The two-choice trials whereby all candidate bait types are tested against each other, as was done for the four bait types used in Mexico, were more discriminating than the elimination-type test protocol used for the confined dogs. However, the total number of all possible paired-bait combinations rapidly increase with an increase in the number of bait types to be tested. For example, if ten different bait types were compared with each other, this would result in 45 possible combinations, obviously an excessive number of trials to conduct. Efforts to decrease the cost and time required for comparing candidate formulations by reducing the number of test dogs, even using replicate trials with the same dogs, unfortunately reduces sample sizes and thereby the sensitivity of the protocol.

Bait trials with Egyptian dogs (1994)

The farmer-owned dogs offered baits were found in doorways, courtyards, alleyways, barnyards or the street adjacent to their owners' dwellings. Virtually all had been fed earlier in the day and prior to the bait trials. Of the first six baits, the DuPont dog-meal bait topically coated with rendered beef tallow and a cheese product (bait A2), and the dog-meal bait coated with poultry byproducts (A6), were the best accepted (Table 5 and Fig. 3). Chi-square analyses indicated differences among the acceptance rates of the first six baits tested ($\chi^2 = 21{,}063$, d.f. = 5, $P = 0{,}001$), but not among the subsequent four supplemental baits ($\chi^2 = 5{,}337$, d.f. = 3, $P = 0{,}142$). There was no detectable difference between acceptance of the beef-tallow/cheese bait (A2) and the chicken-head bait (G) ($\chi^2 = 1{,}148$, d.f. = 1, $P = 0{,}284$), and a difference was marginally detectable in rates of acceptance between the poultry-flavoured bait (A6) and the chicken-head bait (G) ($\chi^2 = 3{,}077$, d.f. = 1, $P = 0{,}079$). The fish-meal polymer bait and the bait provided by Bio-Serv ranked lowest in acceptance, whereas the uncoated DuPont dog-meal bait that served as a control, ranked about midway between all bait types.

Bait-preference rankings of seven baits and coatings that were tested on all three groups of dogs—confined beagles, confined mixed-breed dogs, and free-ranging farmer-owned dogs in Egypt—are shown in Table 4. There was complete agreement in bait preferences between beagles and mixed-breed dogs. Four of the seven bait types and coatings offered to all three groups of dogs (beagles, mixed breeds, and Egyptian dogs) were within one numerical ranking of each other. Although several differences in preference were observed among the three groups (Table 4), the data suggest that the preferences of confined dogs can be used as a reasonable model with which to select baits for field evaluation, even when geographic areas and cultural attributes differ markedly.

Considerable daily variation in the percentage (31–87 %) of all bait types and coatings consumed among the 10 test days was experienced. The reason for this variation is unknown; however, it was noted that the skill of local veterinarians in approaching dogs and presenting them with baits differed, as did their ability

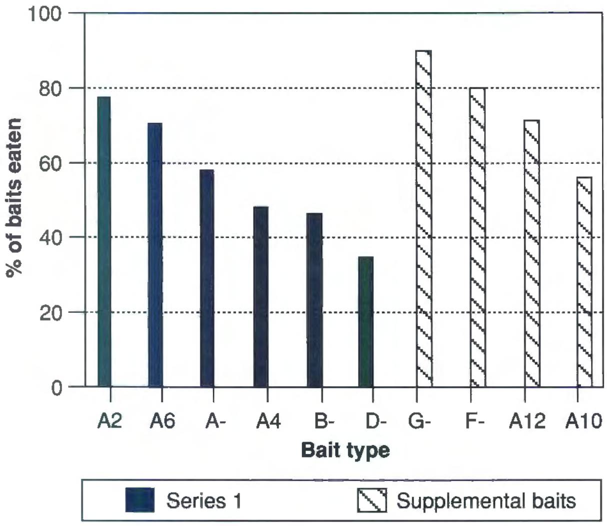

FIG. 3 Bait acceptance by farmer-owned dogs in the Nile River Delta region, Egypt, 1994

TABLE 4 Relative preferece ranking[a] of baits tested in confined beagles and mixed-breed dogs, and in free-ranging Egyptian dogs

Baits/coatings	Beagle	Mixed breed	Free ranging (Egypt)
A6 dog meal/poultry	1	1	2 A4
dog meal/tallow & MFD®	2	2	5 A10
dog meal/egg	3	3	4 A2
dog meal/tallow & cheese	4	4	1 B-
fish-meal polymer	5	5	6 D-
Bio-Serv	6	6	7 A-
dog meal/uncoated	7	7	3

[a] Numerical rankings (1–7) were derived for beagles and mixed-breed dogs by computing the mean percentage of all baits eaten for all trials of a particular bait or coating, and ranking these values with 1 being the highest preference ranking and 7 being the lowest. The number of baits actually eaten of the total number offered was used to determine ranking for the Egyptian dogs

TABLE 5 Acceptance of baits by farmer-owned dogs in northern Egypt

Bait/coating type	% (no. eaten/no. offered)
A2 dog meal/tallow & cheese	77(27/35)
A6 dog meal/poultry	67(24/35)
A-dog meal/uncoated	57(20/35)
A4 dog meal/tallow & MFD®	44(15/34)
B- fish-meal polymer	42(15/36)
D- Bio-Serv	32(12/37)
Supplemental baits	
G- chicken head	88(22/25)
F- beef sausage	80(20/25)
A12 dog meal/dough	70(14/20)
A10 dog meal/egg	56(14/25)

to control the numbers and proximity of curious onlookers, both children and adults, who observed the research team going from house to house. Both the number and proximity of onlookers were directly related to the degree to which dogs became frightened and fled or refused to eat baits. Such procedural differences suggest that a more uniform and consistent method of team selection, a reduction in the number of participants and onlookers, and further standardization of the test protocol are needed to minimize variation in results. Differences in human and dog populations among the three test towns did not appear responsible for the daily variations in bait uptake.

The comparison between one- and two-choice preference tests in Mexico demonstrated greater sensitivity when the two-choice test was used. Therefore, whenever possible and provided field conditions permit, a two-choice preference test should be considered the technique of choice, at least where the number of different bait types to be compared are few. Obviously, some situations may not be conducive to a two-choice test, e.g. throwing baits to free-ranging, unowned dogs, or street dogs unaccompanied by their owners.

It was encouraging that a mass-produced bait (A2) was accepted at almost the same rate as was the chicken-head bait; however, costs associated with their manufacture may limit use. Assuming a manufactured bait continues to show high levels of acceptance in other developing countries where dog rabies is a problem, lowered costs might be achieved by establishing a regional bait-production facility with setup and operating costs shared by several countries, or with funds contributed by private foundations or international agencies.

Even though several bait types were eaten by a high percentage of dogs, this study did not seek to estimate probable levels of vaccine ingestion. Therefore field trials in which baits are used that contain one or more biomarkers (Bachmann *et al.* 1990) as placebo vaccine, should be undertaken. Toward this end, more data are needed to demonstrate to what extent and under what conditions oral vaccination of dogs can enhance or replace conventional parenteral vaccination. Studies summarized by the WHO (1991, 1993, 1994) suggest that supplemental use of oral vaccines in conjunction with parenteral vaccination may be necessary in many countries in order to reach a sufficiently high percentage of the dog population. In the absence of more research, however, the extent to which the oral rabies vaccination of dogs may control the disease, still remains unknown.

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