EVALUATION OF BAITS FOR DELIVERY OF ORAL RABIES VACCINE TO DOGS IN GUATEMALA

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Abstract. Acceptance of oral baits by dogs was evaluated in Guatemala. Eight bait matrix/attractant combinations were produced using commercial materials available in the United States. Two baits were produced using local materials in Guatemala. All baits included a plastic sachet that contained a placebo vaccine (water). Bait trials were conducted February–April, 2002, at five sites using 261 dogs. Bait acceptance ranged from 50.0% to 87.1%, and the combined proportion of sachets either swallowed or punctured ranged from 23.1% to 83.9%. The four bait types with the highest acceptance by dogs were the wax-coated sachet coated with poultry oil and poultry meal (87.1%), the dog meal polymer coated with poultry oil and poultry meal (82.8%), the dog meal polymer coated with poultry oil and poultry meal (77.4%), and the chicken head bait (77.8%). These four bait types were accepted most often as determined both by consumption and combined proportion of sachets swallowed or punctured ($P = 0.0001$). Future trials should demonstrate efficacy of oral rabies vaccination in Guatemala based on the use of selected bait matrices and the poultry oil/poultry meal attractant.

INTRODUCTION

Dogs account for approximately 95% of all animal rabies cases reported worldwide (wildlife cases presumably are under-reported) and are responsible for more than 90% of all human cases.1 Dog rabies has been controlled in North America, Europe, and in a number of individual countries via parenteral vaccination and the control of strays.1-2 Infection and transmission by dogs in other parts of the world is due to various factors, including a lack of rabies control programs and large numbers of unvaccinated free-ranging dogs.2,3 Oral rabies vaccines and baits were first developed during the 1970s and 1980s for wildlife and the initial field trials targeted the red fox (Vulpes vulpes).5 Oral vaccination programs have targeted rabies in red foxes in Europe6 and Canada7 and in raccoons (Procyon lotor), gray foxes (Urocyon cinereoargenteus), and coyotes (Canis latrans) in North America.7,8 Advancements in the development of baits and baiting strategies for oral vaccination of wildlife currently are being used to address rabies in dogs, and may be useful in dog rabies control.2,3 Several baits for delivery of oral rabies vaccine to dogs have been tested in various parts of the world,9 and experimental bait and vaccine trials have been conducted in Turkey, Mexico, Tunisia, South Africa, Sri Lanka, Thailand, Zimbabwe, and The Philippines.2,10-12 However, further studies on bait delivery systems are needed to design safe and cost-effective bait delivery systems,2 and no bait is generally accepted for use in Latin America. The objective of this project was to develop an oral rabies vaccine bait for dogs in Guatemala. Guatemala was chosen for this study based on local governmental support for the project, and the presence of confirmed reports of rabies in dogs in the country.13 Development of an accepted bait and attractant combination in Guatemala will result in its potential availability for use in oral rabies vaccination of dogs in Latin America.

MATERIALS AND METHODS

Baits. Three commercial bait matrices were evaluated, i.e., dog food polymer, fish meal polymer, and a plastic sachet. The dog food polymer and fish meal polymer matrices14 used were the standard baits produced by Bait-Tek, Inc. (Beaumont, TX) for use in current oral rabies programs in the United States. The plastic sachet15 was produced by Merial, Ltd. (Athens, GA). All three bait matrices included the same plastic sachet.

Attractants were added to the bait matrices to increase attractiveness of the baits to dogs. Attractants used were as follows: cheese powder (Cheese Plus; International Ingredients Corporation, St. Louis, MO), beef tallow (Griffin Industries, Ellenville, GA), poultry oil (American Proteins, Inc., Cumming, GA), and poultry meal (Poultry Biodigest; Bioproducts, Inc., Louisville, KY).9,14

Two additional baits were produced using the above plastic sachet and chicken heads10 and pig intestine11 purchased in local markets in Guatemala. A total of 10 bait matrix/attractant combinations were evaluated in this study. The 10 bait matrix/attractant combinations evaluated are described below, including methods of production for each bait.

Bait A was a dog food polymer bait coated with poultry oil and poultry meal. Poultry oil was heated to the point that baits could be dipped in the oil. The oil-coated baits were then placed in a plastic bag containing poultry meal and shaken in the meal to coat the baits with the meal. Averages of 1.58 grams of poultry oil/bait and 3.69 grams of poultry meal/bait were used.

Bait B was a dog food polymer bait coated with beef tallow and cheese powder. Beef tallow was heated to the point that baits could be dipped in the tallow. The tallow-coated baits were then placed in a plastic bag containing cheese powder and shaken in the powder to coat the baits with the cheese powder. Averages of 1.09 grams of beef tallow/bait and 1.54 grams of cheese powder/bait were used.

Bait C was a fish meal polymer bait coated with poultry oil and poultry meal. Poultry oil was heated to the point that baits could be dipped in the oil. The oil-coated baits were then placed in a plastic bag containing poultry meal and shaken in the meal to coat the baits with the meal. Averages of 1.16 grams of poultry oil/bait and 3.69 grams of poultry meal/bait were used.

Bait D was a fish meal polymer bait coated with beef tallow and cheese powder. Beef tallow was heated to the point that
baits could be dipped in the tallow. The tallow-coated baits were then placed in a plastic bag containing cheese powder and shaken in the powder to coat the baits with the cheese powder. Averages of 1.98 grams of beef tallow/bait and 1.81 grams of cheese powder/bait were used.

Bait E was a sachet with poultry oil and poultry meal. Scheel wax (Scheel Corp., Brooklyn, NY) was heated until melted, the sachets were dipped in the wax, and the wax coating was allowed to dry. Moore & Munger wax (Moore & Munger, Inc., Shelton, CT) was heated until melted and 5% poultry oil by volume was added to the wax and stirred. The sachets coated with Scheel wax were then dipped in the second wax and allowed to dry. After drying, the wax-coated sachets were dipped in melted poultry oil, placed in a plastic bag containing poultry meal, and shaked in the meal to coat the baits with the meal. The average weights of the Scheel and Moore & Munger wax coatings were 0.60 grams/bait and 0.85 grams/bait, respectively. The average weight of the poultry oil and poultry meal coating was 1.20 grams/bait.

Bait F was a sachet with beef tallow and cheese powder. Scheel wax was heated until melted, the sachets were dipped in the wax, and the wax coating was allowed to dry. Moore & Munger, Inc. wax was heated until melted and 5% beef tallow by volume was added to the wax and stirred. The sachets coated with Scheel wax were then dipped in the second wax and allowed to dry. After drying, the wax-coated sachets were dipped in melted beef tallow, placed in a plastic bag containing cheese powder, and shaken in the powder to coat the baits with the cheese powder. The average weights of the Scheel and Moore & Munger wax coatings were 0.70 grams/bait and 1.08 grams/bait, respectively. The average weight of the beef tallow and cheese powder coating was 1.33 grams/bait.

Bait G was a dog food polymer bait with no added attractants. This bait matrix was used as produced by Bait-Tek, Inc.

Bait H was a fish meal polymer bait with no added attractants. This bait matrix was used as produced by Bait-Tek, Inc.

Bait I was a sachet with a chicken head. Chicken heads included the debeaked head and neck. Chicken heads were boiled in water for five minutes. A plastic sachet was inserted under the skin of the neck and secured using monofilament line.

Bait J was a sachet with pig intestine. Segments of the small intestine of a pig were washed, the material was removed from inside the intestine, and the intestine was flushed with water. The intestines were then cut into sections of approximately 10 cm in length, and boiled in water for five minutes. A plastic sachet was inserted into each segment of intestine and the ends of each segment were sewn closed using monofilament line.

All 10 bait matrix/attractant combinations included a plastic sachet. This is the same sachet that contains the rabies vaccine in oral rabies vaccination programs in the United States, but the sachets used in our study were filled with water. The sachet was incorporated into the fish meal and dog meat polymer baits. The same sachet was used in production of the wax-coated sachet baits and was included in the chicken head and pig intestine baits.

**Bait trials.** Bait trials were conducted February–April 2002 in five geographic areas in Guatemala. One bait trial was conducted using feral dogs being held at a local humane society in Sumpango, Chimaltenango on February 20, 2002. Two trials involved privately owned dogs brought to public rabies vaccination clinics offered by the Ministerio de Salud Pública in Yupiltepeque, Jutiapa on February 21, 2002 and in Mixco, Guatemala, on March 16–17, 2002. Baits also were offered to privately owned dogs during a house-to-house rabies vaccination campaign in rural areas of Jutiapa on March 12, 2002. The fifth bait trial involved dogs brought to a clinic for distemper vaccination in Patulul, Suchitepequez on April 30, 2002. In all cases, baits were placed on the ground near the dog with the permission of the owner, and the dog allowed to investigate the bait.

Baits were identified by letter code so that the specific bait matrix/attractant combination was not discernable during data collection and analysis, and offered to dogs sequentially. Obvious bait types, e.g., chicken heads, were identifiable during the bait trial, but not during the analysis. Each dog was offered one bait and allowed up to five minutes to consume the bait. If the dog did not attempt to consume the bait, the bait was discarded. If the dog did consume the bait, qualitative data as to whether the dog chewed and/or swallowed the bait were collected. If the dog consumed part of the bait and discarded the plastic sachet, data on whether the sachet was punctured were collected. Differences in proportions of baits accepted were evaluated by chi-square analysis using Epi-Info Version 6 (Centers for Disease Control and Prevention, Atlanta, GA).

**RESULTS**

Tests of the 10 bait types were conducted with 261 dogs. The results of the tests are shown in Table 1. Acceptance of the bait matrix/attractant combinations ranged from 50.0% to 87.1%, as measured by the proportion of dogs that consumed a given bait type. A chi-square analysis indicated significant variation in acceptance among the 10 bait types ($P = 0.01$). The maximum proportion of dogs that could have been vaccinated, as measured by the combined proportion of plastic sachets either swallowed or punctured, assuming the swallowed sachets were punctured, ranged from 23.1% to 83.9%. The four bait types with the highest proportions of acceptance were the plastic sachet with poultry oil and poultry meal (87.1%), the dog meal polymer with poultry oil and poultry meal (82.8%), the fish meal polymer with poultry oil and poultry meal (77.4%), and the chicken head (77.8%). These four bait types ranked highest both by consumption and proportion of sachets swallowed or punctured. A chi-square analysis of these combined four bait types versus all other bait types indicated a higher level of acceptance ($P = 0.0001$).

**DISCUSSION**

Bait preference by dogs has been evaluated using various baits in different parts of the world, and these studies have documented regional differences in bait acceptance. Bait types preferred include dog biscuits in Mexico,\textsuperscript{16} chicken heads in Egypt\textsuperscript{14} and Tunisia\textsuperscript{17,18} the Köfte bait (minced meat with bread crumbs) in Turkey,\textsuperscript{19,20} and boiled pig intestines in the Philippines.\textsuperscript{21} Additional bait studies were reviewed by Limhart.\textsuperscript{7} Because of differing test methodologies, bait types, and cultural environments, comparisons among these field tests are tenuous. In our study in Guatemala we found that poultry flavored baits were accepted most often.
This included baits with poultry flavors impregnated into the bait (coated sachet), coating the bait (dog meal polymer and fish meal polymer), or as the bait itself (chicken head).

Both manufactured baits and locally produced baits are accepted to different degrees by dogs in different regions, but costs and factors related to the process of producing the baits, availability of bait materials, import/export restrictions, and ease of handling in field situations also will be important relative to selection of baits for oral vaccination programs. Both manufactured and locally produced poultry-type baits were highly acceptable to dogs in our study. However, the locally produced chicken head baits were less favored by field personnel due to difficulties in handling and storage of the baits in the field where refrigeration or ice were not available. Based on the results of our field trials and the above factors, the most efficacious bait for use in Guatemala and Latin America may be the coated sachet with poultry oil and poultry meal, but produced using locally available poultry oil and poultry meal where available.

The coated sachet has not been previously tested for use with dogs, and this study does not provide data on the proportion of dogs that would have been vaccinated because vaccine was not included in the baits. However, the poultry oil/poultry meal coated baits provided for the highest proportion of baits consumed, and for the highest proportion of sachets punctured when the sachet was discarded by the dog and could therefore be examined. Future tests should be conducted in Latin America using bait matrices that contain rabies vaccine and the poultry oil and poultry meal attractants. These studies would be used to determine the proportion of dogs vaccinated using the different bait matrices, and demonstrate efficacy of oral rabies vaccination for dogs in Guatemala.

Table 1

<table>
<thead>
<tr>
<th>Bait*</th>
<th>Consumed bait</th>
<th>Swallowed sachet</th>
<th>Discarded sachet</th>
<th>Discarded and punctured</th>
<th>Swallowed and punctured**</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>27/31 (87.1%)</td>
<td>13/31 (41.9%)</td>
<td>14/31 (45.2%)</td>
<td>13/14 (92.9%)</td>
<td>42.0%</td>
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<tr>
<td>A</td>
<td>24/29 (82.8%)</td>
<td>3/29 (10.3%)</td>
<td>21/29 (72.4%)</td>
<td>18/21 (85.7%)</td>
<td>62.0%</td>
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<tr>
<td>C</td>
<td>24/31 (77.4%)</td>
<td>6/31 (19.4%)</td>
<td>18/31 (58.1%)</td>
<td>15/18 (83.3%)</td>
<td>48.4%</td>
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<tr>
<td>F</td>
<td>16/26 (61.5%)</td>
<td>6/26 (23.1%)</td>
<td>10/26 (38.5%)</td>
<td>8/10 (80%)</td>
<td>30.8%</td>
</tr>
<tr>
<td>G</td>
<td>16/26 (61.5%)</td>
<td>5/26 (19.2%)</td>
<td>14/26 (53.8%)</td>
<td>4/14 (28.6%)</td>
<td>15.4%</td>
</tr>
<tr>
<td>I</td>
<td>14/18 (77.8%)</td>
<td>10/18 (55.6%)</td>
<td>4/18 (22.2%)</td>
<td>1/4 (25.0%)</td>
<td>5.6%</td>
</tr>
<tr>
<td>D</td>
<td>20/30 (66.7%)</td>
<td>7/30 (23.3%)</td>
<td>13/30 (43.3%)</td>
<td>8/13 (61.5%)</td>
<td>26.6%</td>
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<td>F</td>
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<td>6/26 (23.1%)</td>
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</tr>
<tr>
<td>B</td>
<td>14/26 (53.8%)</td>
<td>5/17 (29.4%)</td>
<td>9/17 (52.9%)</td>
<td>5/9 (55.6%)</td>
<td>31.8%</td>
</tr>
</tbody>
</table>

* Bait E = sachet with poultry oil and poultry meal; Bait A = dog food bait matrix with poultry oil and poultry meal; Bait C = fish meal bait matrix with fish meal polymer; Bait F = dog food bait matrix with fish meal polymer; Bait I = sachet with chicken head; Bait J = fish meal bait matrix with fish meal and cheese powder; Bait E = sachet with fish meal and cheese powder; Bait G = dog food poultry bait with no added attractants; Bait J = sachet with pig intestines; Bait H = fish meal polymer bait with no added attractants; Bait B = dog food bait matrix with fish meal and cheese powder.
† Bait was consumed in part or in whole.
‡ Plastic sachet was swallowed.
§ Plastic sachet was discarded.
¶ Discarded sachet was punctured.
** Proportion of sachets either swallowed or punctured (proportion of sachets swallowed + proportion of sachets discarded and punctured).

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