

## Bait formulation and distribution for oral rabies vaccination of domestic dogs: an overview

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### ABSTRACT

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Current efforts to develop oral rabies vaccine baits for domestic dogs are reviewed and new materials (bait matrices, additives, vaccine containers, vaccine modification) for improving bait acceptance and vaccine delivery are suggested. Methods that have been used to evaluate the food or bait preferences of confined and free-ranging animals are summarized, as are the guidelines for bait distribution compiled by the World Health Organization.

### INTRODUCTION

Dog rabies in North America and Europe has been controlled largely with parenteral vaccines and the control of strays; less than 5% of all cases are in this species (Fekadu 1991). However, such is not the case worldwide where about 95% of all animal rabies cases are in dogs (Fekadu 1991) and they are responsible for over 99% of all human cases of the disease (Joshi & Bögel 1988). The large numbers of unvaccinated free-ranging dogs, both owned and ownerless, are a major factor contributing to the lack of effective rabies control in many of the tropical countries (Joshi & Bögel 1988; WHO 1988b). Human societal and cultural factors as well as the dynamics, ecology, and behaviour of dog populations all contribute to the problem (Wandeler, Budde, Capt, Kappeler & Matter 1988).

Recognizing a need to better understand the disease in dogs, guidelines for dog ecology studies were compiled by the World Health Organization (WHO 1984; 1988a; 1990). Studies have been undertaken in various countries to estimate dog densities and to

understand their dynamics by acquiring data on reproductive rates, age structure and population turnover. Statistics also have been compiled about the cultural aspects of human-dog relationships, characteristics of dog ownership and other sociological parameters that might contribute to planning and implementing dog rabies control programmes (Rangel 1981; Beran 1982; 1985; Ben Osman 1985; Wandeler 1985; Artois & Ben Osman 1986; Matter 1987; Wandeler *et al.* 1988; Oboegbulem & Nwakonobi 1989; Brooks 1990; Beran 1991). These studies and others have provided us with the conceptual framework within which to investigate and implement the oral vaccination of the species.

Much effort has been directed at the oral vaccination of wildlife, especially the red fox (*Vulpes vulpes*), beginning in the mid-1960s (Winkler 1992). At one point, between 1972 and 1977, the technique was being investigated by at least 15 groups of investigators in nine different countries (Debbie & Bögel 1988). Use of vaccine baits for immunizing red foxes subsequently has become routine in several European

countries (Winkler 1992). Although the development of parenteral dog vaccines has also received much attention, only recently has effort been aimed at oral vaccination of this animal. The WHO has provided research guidelines (WHO 1988b; 1989; 1991; 1992) that encompass vaccine efficacy, safety and specificity, planning and organization of field trials, use of oral vaccines in both urban and rural areas, accessibility of dogs for vaccination, potential field study sites, standardization of protocols and the relationship between oral vaccination and dog ecology and behaviour. The guidelines also include development of vaccine baits, techniques for evaluating their efficacy and baiting strategies to maximize bait ingestion by targeted dog populations.

This paper reviews the current status of vaccine baits, suggests ways to increase acceptance by improving bait matrices, additives and vaccine containers, discusses bait preference testing protocols and summarizes methods used to assess the efficacy of vaccine baiting strategies for dogs.

## PRESENT STATUS OF VACCINE BAITS

Historically, food baits were used to poison wild fur-bearers for their pelts (Russell 1967; Dannenfeldt 1982; Snetsinger 1983) and later for delivering toxicants to wild carnivore populations that carried rabies (Ballantyne & O'Donoghue 1954; Coccozza & Malaga Alba 1962; Lewis 1975; Debbie 1991; Muller 1992). The systematic evaluation of food baits and odour attractants began in the 1960s concurrent with interest in using orally administered antifertility agents for reducing coyote (*Canis latrans*), red fox and striped skunk (*Mephitis mephitis*) populations (Balser 1964; Linhart, Brusman & Balser 1968; Brusman, Linhart, Balser & Sparks 1968; Nelson & Linder 1972). These initial efforts were followed by more comprehensive work that included development of experimental bait and attractant test protocols for both confined and free-ranging carnivores. Such studies included comparative tests of lures and baits traditionally used by trappers and animal damage control specialists, synthetic odour attractants based on identification of the chemical fractions in carnivore urine, anal gland secretions and attractants produced by fermentation of various materials of animal origin (e.g. Linhart, Dasch, Roberts & Bullard 1977; Timm, Howard, Monroe, Teranishi & Murphy 1977; Rough-ton & Bowden 1979; Turkowski, Popelka, Green & Bullard 1979; Teranishi, Murphy, Stern, Howard & Fagre 1981; Fagre, Butler, Howard & Teranishi 1981; Bullard, Turkowski & Kilburn 1983; Turkowski, Popelka & Bullard 1983). Concurrent and subsequent investigations in the United States, Canada and Western Europe focused on administering oral rabies vaccines to wildlife vectors of the disease using various bait materials. This work has been reviewed (Schneider, Cox, Muller & Hohnsbeen 1988; Wan-

del 1991; Kappeler 1992; Winkler 1992; Bögel, Meslin & Kaplan 1992).

The recent and quite limited efforts to test efficacious baits for administering oral vaccines to dogs have borrowed from the earlier wildlife studies mentioned above and the few baits evaluated thus far have largely been those previously developed for red foxes and raccoons (*Procyon lotor*). Winkler & Baer (1975) and Baer (1976) were apparently the first investigators to develop a bait for orally vaccinating dogs; they inserted a sealed plastic straw containing rabies vaccine into a commercially available sausage. In Zimbabwe, Perry and co-workers (Perry, Brooks, Foggin, Bleakley, Johnston & Hill 1988) used a dog bait that had been initially developed for red foxes and raccoons. It consisted of a polyurethane sponge cube impregnated with a liquid placebo vaccine (egg yolk, molasses in water and dye marker) and was placed in the field along with a fermented odour attractant to enhance bait discovery. Baer, Brooks & Foggin (1989) used preformed cigar-shaped baits of boiled and deep-fried cornmeal to administer liquid canine adenovirus vaccines to confined dogs (such vaccine viruses were of interest as potential vectors for a recombinant rabies vaccine). Matter, Kharmachi, Hadded, Ben Youssef & Seghaier (unpublished data) used chicken heads as a bait for dogs in suburban areas of Tunisia. Also in Tunisia, Kharmachi, Haddad & Matter (1992) used household dogs to test four bait types; a sausage bait made of donkey meat and cooked rice, a Du Pont polymer fish meal bait, a chicken head bait and a polyurethane sponge bait inside a plastic packet that also contained a fermented odour attractant. Three of the above four baits had been originally developed for red foxes and raccoons. Frontini, Fishbein, Garza Ramos, Flores Collins, Balderas Torres, Quiroz Huerta, Gamez Rodriguez, Belotto, Dobbins, Linhart & Baer (1992) tested four different candidate dog baits in rural Mexico, all of which were developed for wildlife. Two baits consisted of cylindrical corn, milk and egg batter-coated polyurethane sponge baits (Linhart, Blom, Dasch, Roberts, Engeman, Esposito, Shaddock & Baer (1991) either deep-fried in corn or fish oil and then air-dried. The other two baits were a Du Pont polymer fish meal bait (Hanlon, Hayes, Hamir, Snyder, Jenkins, Hable & Rupprecht 1989) and a Canadian tallow/wax chicken-flavoured bait containing a blister pack (Bachmann, Bramwell, Frazer, Gilmore, Johnston, Lawson, MacInnes, Matejka, Miles, Pedde & Voigt 1990). A commercially produced dog biscuit was used as a standard or control food item. Bait ingredients and results of the above tests are shown in Table 1.

As may be seen, only modest and quite recent efforts have been aimed at developing and testing vaccine baits for dogs; such baits have largely been those previously formulated for wild carnivores. Future

TABLE 1 Baits tested for delivery of oral rabies vaccine to domestic dogs

Bait types tested	Size	Type of test	Baits bitten or chewed by dogs(%)	Baits completely ingested (%)	Dogs marked with Placebo <sup>a</sup> vaccine (%)	Source and location of study
Slim Jim <sup>b</sup> sausage	? x 13 cm	Laboratory dogs	-	-	-	Winkler & Baer 1975, Baer 1976
Polyurethane sponge cube in plastic sachet with fermented attractant in outer bag	2 x 3,5 x 5 cm	Farms: free-ranging dogs	79(65/820)	-	25(138/553)	Perry <i>et al.</i> 1988 (Zimbabwe)
Cooked preformed corn meal deep-fried in corn oil	Cigar-shaped cylinder 10 cm long	Farm-owned dogs	-	100(11/11)	-	Baer <i>et al.</i> 1989 (Zimbabwe)
Chicken head	-	Suburban: owned dogs unowned dogs	- - -	- 78(?) 56(10/18)	- 78 56	WHO 1991 (Tunisia)
Cylindrical polyurethane sponge containing corn meal, egg, milk and deep-fried in: corn oil  fish oil	1,5 x 5,5 cm	Rural towns: owned dogs	-	-	-	Frontini <i>et al.</i> 1992 (Mexico)
	1,5 x 5,5 cm		88(45/51)	67(34/51)		
			85(33/39)	69(27/39)		
Du Pont fish meal polymer	2 x 3 x 5 cm	Rural towns: owned dogs	90(43/48)	50(24/48)	-	Frontini <i>et al.</i> 1992 (Mexico)
Canadian blister pack (wax)	2 x 3,5 x 3,5 cm	Rural towns: owned dogs	44(17/39)	10(4/39)	-	Frontini <i>et al.</i> 1992 (Mexico)
Small dog biscuit (control)	1 x 2,3 x 4,5 cm	Rural towns: owned dogs	97(171/176)	88(155/176)	-	Frontini <i>et al.</i> 1992 (Mexico)
Sausage of minced donkey meat and cooked rice	7-10 cm long	Owned dogs	56(28/50)	-	46(13/28)	Karamachi <i>et al.</i> 1992 (Tunisia)
Du Pont fish meal polymer	2 x 3 x 5 cm	Owned dogs	80(40/50)	-	78(31/40)	Karamachi <i>et al.</i> 1992 (Tunisia)
Chicken head	-	Owned dogs	96(48/50)	-	98(47/48)	Karamachi <i>et al.</i> 1992 (Tunisia)
Polyurethane sponge cube in plastic sachet with fermented attractant <sup>c</sup>	?	Owned dogs	66(33/50)	-	30(10/33)	Karamachi <i>et al.</i> 1992 (Tunisia)
0,5 large dog biscuit	1,5 x 5,0 x 5,5 cm	Rural towns: owned dogs	-	81(108/134)	-	Linhart <i>et al.</i> (unpublished data Mexico)
Cylindrical polyurethane sponge containing corn meal, egg, milk and deep-fried in corn oil	1,5 x 5,5 cm	Rural towns: owned dogs	-	84(111/133)	-	Linhart <i>et al.</i> (unpublished data Mexico)

TABLE 1 (continued)

Bait types tested	Size	Type of test	% of baits bitten or chewed by dogs	% of baits completely ingested	% of dogs marked with Placebo <sup>a</sup> vaccine	Source and location of study
Same as above but shorter length	1,5 x 3,0 cm	Rural towns: owned dogs	–	83(108/130)	–	Linhart <i>et al.</i> (unpublished data Mexico)
Length of beef hot dog dried and hardened	1,5 x 4,5 cm	Rural towns: owned dogs	–	77(104/136)	–	Linhart <i>et al.</i> (unpublished data Mexico)

<sup>a</sup> Numerals in parenthesis are % of dogs positive over total dogs checked

<sup>b</sup> Attractant consisted of fermented meat, offal, fish, blood, cheese and yeast; 5 ml placed in outer bag

<sup>c</sup> Attractant consisted of fermented minced meat, eggs, yoghurt, fish and cheese

vaccine bait development for dogs, it is believed, should include some of the considerations outlined below.

#### FUTURE BAIT DEVELOPMENT

The World Health Organization criteria for dog vaccine baits (WHO 1988b) provide a useful reference point for future bait development and thus are abstracted below. Baits should:

- contain a vaccine in sterile form and be freezable;
- be protective of vaccine with respect to temperature, rainfall and ultraviolet light;
- be immediately attractive to dogs to ensure rapid ingestion;
- be unattractive to humans (children) and non-target species;
- incorporate vaccine as homogenous as possible to ensure its ingestion;
- carry a biomarker in attractant and/or vaccine;
- be shaped to allow easy ingestion by all sizes and ages of dogs;
- include a vaccine labelling or identification system, if required;
- be inexpensive, contain locally available products known to be attractive to dogs and be capable of production under local conditions;
- be accepted in different societies and circumstances of urban and rural dog ecology and behaviour.

With respect to developing more efficacious, selective and cost-effective vaccine baits (for both dogs and wildlife), bait components may be considered as matrices, additives, vaccine containers and vaccine modifications.

#### Bait matrices

Thus far, materials for formulating vaccine baits have consisted primarily of meat, tallow, waxes, oils and fish meal. However, a number of inexpensive grains and other animal by-products are available from the livestock and poultry feed industries and have not been considered for use as vaccine bait matrices. For example, poultry feed mills formulate feed pellets of bone and meat scrap meal, ground corn, soybean meal and poultry by-products. The pet food industry has a long history of evaluating food products for dogs and cats; to my knowledge their expertise has not yet been tapped. Despite the proprietary nature of dog food manufacture, industry R & D staffs may be willing to offer suggestions, provide prototype baits and perhaps ultimately to produce custom baits for oral vaccination.

Food industries that manufacture flours and meals for human consumption are another potential source. For example, commercial corn bread and corn dog batter mixes were successfully used to formulate raccoon baits (Linhart *et al.* 1991). A number of freeze-dried vegetable, poultry and animal products are now available and might be useful; dried milk and freeze-dried eggs are certainly two that should be included in experimental formulations. Prototype mongoose baits have been formulated from freeze-dried eggs and with other materials were well accepted by free-ranging mongooses (Linhart, Creekmore, Corn, Whitney, Snyder & Nettles 1993). The knowledge and formulation expertise within the confectionery industry has not yet been utilized as a source of potential bait materials. Help from confectioners might be especially useful as dogs (and wild canids) are known to readily ingest natural and manufactured food items containing sugars.

The aquaculture industry is another possible source of information; most commercially produced fish in the southern United States are fed pellets made from inexpensive food by-products. An unique requirement

of such feed is that pellets must remain intact in the water until ingested. Few people are likely to be aware that the origin of the so-called Wistar, Merieux, or Du Pont fish meal polymer vaccine bait that contains a patented waterproofing polymer (Smith & Daigle 1988), used both in the United States and Europe, was an attempt by the E.I. Du Pont de Nemours & Company to find other markets for their fish food product.

Use of synthetic materials, especially those of plastic origin, may produce more durable, stable and uniform vaccine baits. For example, polyurethane sponge has been used as a matrix for red fox (Johnston & Lawson 1987), raccoon (Rupprecht, Dietzschold, Koprowski & Johnston 1987; Linhart *et al.* 1991), dog (Perry *et al.* 1988; Frontini *et al.* 1992; Kharmachi *et al.* 1992) and mongoose (Linhart *et al.* 1993) vaccine baits. Another synthetic material involves containment of liquids within a foam substrate and micropackaged particle composite until released by external pressure exerted on the material (Hermann 1989). Finally, warm water fishing lures in the United States are commonly molded or extruded using a liquid plastic that dries to a soft consistency; many now contain a high percent of "natural" food products such as fish oil and meals. Such a material and fabrication process may have potential for making vaccine baits provided available plastics are nontoxic. The use of synthetic materials for bait matrices has hardly been explored. One might envisage a completely synthetic vaccine delivery device that is uniform, nontoxic, attractive to target species, durable in various environmental circumstances and that contains the vaccine without the need for a separate vaccine container.

### Bait additives

Surface coatings, such as slurries on bait matrices or substances incorporated into bait substrates, can increase target animal discovery and consumption of baits, can make them more selective, or can repel inquisitive humans. A variety of largely unexplored options may have potential.

Until recently, most of what we knew about odour and gustatory attractants for wild carnivores (including canids) came from fur trappers and animal damage control specialists who, through trial and error, developed a wide variety of lure and bait formulations. Such formulations generally consisted of mixtures of common animal products or plant extracts, most of which are available from trapper supply houses. In turn, supply houses obtain many of their ingredients from international fragrance and flavour companies that supply their products to the food industries (livestock, pet, and human) and manufacturers of perfumes, toiletries and a wide variety of other products that use smell or taste to entice animals or we hu-

mans. While the origin of many of these products is still from animal and plant-derived extracts, a large number are now synthetic in origin, chemically stable and can be obtained in either water- or oil-soluble formulations. Examples are the synthetic beef, pork, lamb, liver, cheese, and chicken flavours used by the animal feed and food industries.

One important ingredient of some trapping lures is the excised anal glands of furbearers. This material is an effective odour attractant because the short-chain volatile fatty acids produced by such glands are secreted into the environment and are a basis for odour communication between free-ranging carnivores, including wild and domestic dogs. The subject has attracted the attention of behavioural biologists and animal psychologists and the study of chemical signals and their role in communication has led to identification of the volatile fatty acids in anal gland secretions (e.g. Albone & Fox 1971; Albone, Gosden, Ware, Macdonald & Hough 1978—for the red fox; and Preti, Muetteties, Furman, Kennelly & Johns 1976—for the dog and coyote). Of interest to us are similarities between the compounds present in anal glands, those associated with aerobic and anaerobic decomposition of carrion and those between canids and their prey (Bullard 1982; 1985). Formulations based on these compounds are termed "synthetic" attractants and are among the most attractive to wild canids (Teranishi *et al.* 1981; Scrivner, Teranishi, Howard, Fagre & Marsh 1987; Phillips, Blom & Engeman 1990). They also should be useful for attracting domestic dogs but so far it is unknown to what extent and under what circumstances olfactory attractants will enhance bait discovery and ingestion by this species.

While odour attractants (e.g. volatile fatty acids) can be used to enhance bait discovery they will not necessarily increase bait ingestion. Care must be taken not to use too great an amount as some may repel canids when in close proximity or cause them to roll on rather than eat baits. However, when used in small amounts, they are much more easily handled than the slurries previously used and made from natural food materials. They are inexpensive and can be ordered from chemical supply companies and can also be formulated into impervious carriers that provide for their timed release regardless of prevailing weather conditions (Turkowski *et al.* 1983).

Some bait surface coatings that can be used to enhance carnivore bait uptake are fish, blood and liver meals, powdered or granular sugars and various cheese or cheese-based products. Some are applied simply by shaking baits in a bag containing the coating while others may require a carrier to distribute and affix them to the bait. Some examples of commonly used solvents or carriers are corn, peanut or fish oils, propylene glycol, corn starch and water, or

Rhoplex, the latter a liquid latex that dries and adheres to a bait material. Another is diglyme in which oil and water soluble compounds are both soluble (Scrivner, Howard & Teranishi 1985). Carriers must be used with care because some may mask or alter additive odours or taste enhancers.

Baits may be modified to make them waterproof, to extend their field life, or to make them unattractive to humans. For example, baits can be made water resistant by dipping or spraying them in a mixture of corn oil, melted paraffin and beeswax, or distributing them in light weight plastic bags to protect them from rain and moisture. Baits made from some materials are subject to moulds and fungi and thus antimicrobial compounds such as sodium benzoate or thiabendazole should be included in the formulation. The oils used in some baits may turn rancid over time and alter both odour and taste qualities; an antioxidant such as ethoxyquin may be required for such problems. Trapper supply houses, pet food manufacturers and commercial food processors can provide information on ways to preserve and stabilize bait ingredients.

Several possibilities exist for reducing human interference with baits. One involves using odours that attract dogs but are repugnant to humans. Such odours include butyric and valeric acids, present in both carnivore anal glands and carrion (Bullard 1982). (Trappers also use valeric acid in certain lures.) Other odourants that are likely to attract dogs but repel humans include those developed and discussed by Fagre *et al.* (1981) and Bullard (1982) and field tested by Fagre *et al.* (1981), Roughton (1982), Turkowski *et al.* (1983), Jolly & Jolly (1992) and others. Several of the compounds developed by the above investigators elicit biting and chewing responses as do the repugnant smelling baits used on coyote getters or M-44s devices used in North America and southern Africa to deliver sodium cyanide to coyotes and jackals. Other compounds repellent to humans such as cadaverine, putrescine, butylmercaptan (putrid), and dentorium benzoate (Bitrix, bitter-tasting) could be tested to determine their acceptance by dogs.

Obviously, there are a great array of options available for developing inexpensive and effective baits for delivering oral rabies vaccines not only to dogs but also to other carnivore species that carry the disease.

### Vaccine containers

Vaccine containers within baits that would be better accepted by carnivores and consistently and uniformly deliver liquid vaccine into the oropharyngeal region of the mouth are needed, not only for dogs but also for wildlife. Wandeler, Capt, Kappeler & Hauser (1988) stated that, "...foxes often reject vaccine containers incorporated into otherwise texturally homogeneous baits." Dog baittests in a rural area of cen-

tral Mexico revealed that wax ampules and plastic sachets incorporated into different bait types were sometimes poorly penetrated or incompletely consumed. Dogs sometimes separated ampules from baits and varying amounts of placebo vaccine was either retained within containers or lost onto the ground (Frontini *et al.* 1992; Linhart *et al.*, unpublished data). These observations make it clear that better ways are needed to deliver oral vaccines to target species.

Several approaches for improving vaccine containers merit investigation. First, an extensive inquiry to find new and innovative containers should be directed to the pharmaceutical, food packaging, confectionery and retail product packaging industries. This can be achieved by selecting potentially useful commercial firms from such indexes as the Thomas Register in the United States and similar listings in Europe and elsewhere such as Dun and Bradstreet—Guide to Key British Enterprises, Kompass Deutschland: Register of Selected German Industry and Commerce and Henderson's "Current European Directories" which lists industrial and commercial directories by country.

There are other possibilities that may lead to more effective vaccine containers and delivery of contents. For example, because dogs often detect containers within baits and discard them, a means should be found to affix or cement containers within bait cavities. Thus, when dogs consume baits the containers would also have to be ingested. Adding attractive flavours or odours to the waxes and plastics used to fabricate containers is another alternative that should increase consumption. For example, the wax ampules used in raccoon baits (Hanlon *et al.* 1989; Linhart *et al.* 1991) can be made with a wax containing 25% sucrose (W & F Products, Inc., 2299 Kenmore Avenue, Buffalo, NY 14207 USA, personal communication).

Development of a vaccine container that would serve both as container and bait by affixing attractants to its exterior wall also may have potential. A somewhat similar approach was taken by Marsh, Howard, McKenna, Butler, Barnum & Teranishi (1982) who developed a liquid delivery device for coyotes by coating a plastic container with a substance that elicited biting and chewing behaviour. The original device, termed a CLOD ("coyote lure operative device"), was subsequently modified by Fagre & Ebbert (1988) and others who used sweet corn syrup as a carrier that was slowly released from the device when punctured by a coyote.

### Vaccines

I am unaware of any studies that have sought to determine whether dogs respond to the taste of rabies vaccine in a positive, negative, or neutral manner. If dogs were attracted to vaccine as a food item, it's

likely that more complete ingestion would result. While many organic additives to vaccine would adversely affect vaccine potency other flavour enhancers might be suitable. For example, during the late 1970s and 1980s a 7-year effort between Florida State University and the Pet Foods Division, General Foods Corporation, culminated in a series of papers on canine olfaction, taste and feeding (*Neuroscience and Biobehavioral Reviews* 8:167-265, 1984). Among other findings, it was shown that dogs discriminated between certain sugars and sweeteners and that several increased their food consumption. Since sucrose can be used as a vaccine stabilizer and apparently does not adversely affect titre, it may be feasible to enhance vaccine uptake by incorporating it into oral vaccines. Another approach might be to increase the vaccine viscosity by adding an inert material such as glycerol (a vaccine preservative) so that less vaccine is lost during bait ingestion. Such an approach may, however, result in fewer virions coming into contact with the oropharyngeal region and thereby reduce immunogenicity. Finally, injection of vaccine by syringe directly into a bait immediately prior to presentation to a dog might be a feasible procedure. This would eliminate the problems of container, incompatibility of vaccine and bait material, vaccine bait refrigeration and other associated problems.

### BAIT PREFERENCE TESTING

Studies of domestic and wild animal food preferences have encompassed such diverse areas as basic research on anatomy and physiological functions as they relate to odour and taste perception, comparisons of different experimental techniques to distinguish which foods are preferred and field tests to determine the best bait materials for delivering chemicals and biologicals to wild carnivores. These investigations have been conducted by flavour chemists, biochemists, behavioural psychologists and wildlife biologists and may have direct application for developing and testing baits for oral vaccine delivery.

In 1977, the American Chemical Society sponsored a symposium, "*Flavour Chemistry of Animal Foods*" (Bullard 1978). The papers presented ranged from progress in flavour research to applicable methodologies associated with developing palatable foods for domestic pets; the authors represented academia, industry and government. Jacobs, Beauchamp, & Kare (1978) pointed out in their introductory paper that the term "flavour" is an interaction of complex receptors that may include taste, olfactory, vomeronasal and the chemical sense inputs as well as tactile, temperature and proprioceptive cues. They wrote that sensory reception and response have been shaped by environmental pressures during the course of evolution and that compounds in the envi-

ronment have had great influence on survival. For example, many fruits and vegetables contain various sugars and extensive feeding on these foods has resulted in animals responding positively to such substances. Conversely, plants have evolved to produce toxic, bitter tasting substances as protective adaptations and for this reason most animals generally reject compounds that have a bitter taste. Obviously then, basic taste sensations "... have great ecological relevance for many species". The authors further point out that efforts to measure taste and food preference have led to the use of differing experimental paradigms thereby making comparisons difficult or impossible. It is evident that innate behavioral responses to food flavours and food preference testing have important roles in developing vaccine baits for dogs.

Chalupa & Baile (1978) at the above meeting stated that while human taste responses have been categorized into sweet, sour, bitter and salty, animal responses can be better described as preferences, aversions, or indifferences. The implication here, of course, is that we should not extrapolate human taste and odour perceptions to other species such as the dog. Shumake (1978) discussed research findings on bird and mammal food preference behaviour and pointed out that past association or experience with flavours can be as important, if not more so, than their sensory content. He stated that numerous factors influence and determine food preferences (i.e. nutritional contents, physiological, behavioural, ecological and genetic factors and genetic effects of domestication) and that individuals involved in animal food flavour research should be aware of them. Schumake concluded by saying that food flavour familiarity may be the most important factor controlling preferences, but that mammals also "...tend to sample small amounts of any new food item placed in their environment". Smith & Rashotte (1978), in the same symposium, described the behavioural methodology associated with animal food development. They pointed out that animal response and behaviour will vary as a function of the types of tests used to measure food acceptance and divided such tests into four basic types; short term, long term, single food and multiple foods. They discussed the advantages and disadvantages of each type test and wrote that prior experience with a test protocol or food type can profoundly affect the outcome of preference tests.

Although much dog food preference research has been conducted by industry and thus is proprietary and unpublished, nonetheless some published information is of interest to those developing vaccine baits. For example, as early as the mid-1950s, Beidler, Fishman & Hardiman (1955) used rats, guinea pigs, rabbits, cats, hamsters, raccoons and dogs to study and report on differences in species responses to chemical stimuli (various salts) by means of

electrophysiological experiments. Rashotte, Foster, & Austin (1984) compared dog preferences for various foods using two-pan and operant lever-press tests and found that these two test methods did not always lead to the same results and conclusions. Smith, Rashotte, Austin & Griffin (1984) compared dog eating behaviour using single-pan and two-pan tests and found differences in the types of information derived from each. Griffin, Scott, & Cante (1984) using the identical food found discrepancies between dog food preferences when tested in testing kennels and in consumers' homes. All these studies remind us that care should be taken and the advice of experts solicited before designing experimental protocols and conducting dog bait preference tests.

So far, relatively few tests have been conducted to compare dog preferences for different prototype vaccine baits. Investigators have used two types of tests; those with confined dogs or dogs under the control of their owners (i.e. household dogs) and those that tested uncontrolled or free-ranging dogs. Dog bait preferences with owned dogs were conducted by Frontini *et al.* (1992) who obtained information about rural households in central Mexico and then randomly selected dogs that were subsequently presented with one of four experimental baits. Dogs were observed during bait presentation and the amount of bait eaten, total chewing time, number and size of pieces of bait left on the ground, amount of placebo vaccine (dye water) spilled on ground and condition of ampules containing the dye were recorded. A commercially sold dog biscuit was used as a control or reference food material. On a subsequent visit to the same area, Linhart *et al.* (unpublished data) tested four prototype baits and a control (dog biscuit) but randomized the order in which test baits were presented rather than randomizing the dogs assigned to the test. This procedure proved much more rapid and yielded comparable information. We also compared single bait versus paired bait presentations to owned dogs to determine which of the methods was more rapid and better demonstrated bait preferences. Kharmachi *et al.* (1992) tested four different bait types in the Utique and Kalaat El Andalous region of northern Tunisia by systemically visiting households and presenting single baits, always in the same order, to individual dogs as they were encountered in each household. They used 50 dogs per bait type and placed dyes (methylene blue or rhodamine B) in baits which when present in and about the mouth was indicative of probable vaccine delivery.

Perry *et al.* (1988) used unrestrained dogs in eastern Zimbabwe to determine acceptance of two types of sponge baits. Baits containing rhodamine B dye were presented along with a fermented slurry. Following a pilot test on two farms, they baited two areas the day before dogs were brought to parenteral

rabies vaccination clinics. Bait disturbance rates and visual inspection of dogs for evidence of dyes at clinics the following day were indicative of bait uptake and used to determine potential vaccination rates. Free-ranging dog acceptance of a red fox bait ("Tubingen" bait) has been determined by placing 13 bait clusters containing six baits each at dump sites in Tunisia. Baits were placed on sifted earth (tracking stations), left overnight and inspected the following day for signs of animal activity. The absence of baits and presence of animal tracks were used to determine bait disappearance rates and the species that visited stations (WHO 1988b). The above technique was originally developed to evaluate wild carnivore baits and odour attractants, especially for the canids (Linhart & Knowlton 1975; Linhart *et al.* 1977; Albone *et al.* 1978; Roughton & Bowden 1979; Turkowski *et al.* 1979; Allen, Fleming, Thompson & Strong 1989). Linhart *et al.* (unpublished data) randomly offered one of four test baits (plus control biscuit) to individual street dogs in central Mexico in 1990 and recorded the percent of each type eaten or rejected and the fate of water-filled ampules within baits. Results were then compared with one and two choice bait tests of household dogs to determine which was the most effective procedure.

## BAIT DISTRIBUTION

So far, very few field studies have compared different strategies for distributing vaccine baits to dogs. The reasons are probably two-fold. First, interest in orally vaccinating dogs is recent and research efforts as well as funding have been modest and second, no oral vaccine for dogs has as yet been fully tested to resolve questions regarding safety and efficacy under field conditions. However, guidelines for field studies are available, primarily in WHO reports. In a 1988 WHO publication (WHO 1988b), the highest priorities were oral vaccine strains, vaccine baits, biomarkers, bait acceptance, application of ecological data to oral immunization campaigns, public education and community participation. In 1989, WHO (WHO 1989) characterized the different approaches for distributing baits to dogs as:

- aerial distribution (not generally recommended);
- non-specific distribution (e.g. from a moving vehicle such as a bicycle, automobile);
- specific, manual placement without recovery of baits at selected sites (maximizes target and minimizes non-target uptake, especially humans);
- specific manual placement with recovery of baits at selected sites (labour intensive but further improves target species exposure and minimizes human exposure);
- hand-feeding of baits to individual dogs (facilitates identification of vaccinated animals but may increase risk to human handlers).



In subsequent reports (WHO 1991; 1992) WHO outlined the following steps for organizing field trials:

- Select study sites(s).
- Estimate size of dog population.
- Estimate accessibility of dog population (for both parenteral and oral vaccination).
- Determine bait acceptance by using placebo baits containing biomarkers and by:
  - (a) house to house distribution;
  - (b) street or field distribution.
- Compare efficacy of above techniques and, in combination with parenteral vaccination, ensure maximum safety for non-target species, especially humans.
- Determine the optimal strategy under local environmental conditions cultural practices and prevailing dog ecology/behaviour.
- If parenteral vaccination is planned, it should be organized using mobile vaccination centres and dogs should be marked so that unmarked dogs found later can be offered vaccine baits.
- Evaluate overall programme.

Additional specific recommendations included:

- Formulate baits acceptable to dogs but repugnant to people to minimize human handling of baits. Include a reference or control bait with field trials.
- Determine the best combination of parenteral and oral vaccination under local conditions.
- Consider public education and cultural characteristics as an integral part of any vaccination programme.
- Acquire and integrate dog ecology and behavioural data to increase bait acceptance. For example, dogs may refuse to take baits when outside their accustomed home range.
- Compare cost effectiveness of parenteral versus oral vaccination.
- If feasible, use both topical and systemic biomarkers in baits.
- Evaluate alternate methods of bait delivery, i.e. direct to dogs by trained research personal, by local personnel, or by owners.
- Determine the bait densities needed to reach a given percent of the dog population.
- Determine optional time to distribute baits (e.g. when dogs are fed by owners, by time of day, day of week, season of year and weather conditions, non-target disturbance).

- Define under what conditions baits should be left overnight for free-ranging dogs at garbage dumps, slaughter houses, etc.

Most recently, WHO suggested procedures for evaluating dog vaccine bait delivery techniques (Matter 1993; WHO 1993). These guidelines assumed an efficacious bait well accepted by dogs under field conditions. They recommend that tests be conducted in villages where the number of inhabitants and/or households can be determined and that have 5 000–10 000 inhabitants and  $\geq 500$  dogs. Four types of sequential field trials, including specific protocols and equipment lists, were recommended:

**Test 1** This test utilizes a bait with systemic and topical markers and seeks to determine how well dogs accept the bait and the contact rate with a placebo vaccine (i.e. systemic biomarker in bait) based upon analysis of blood sera taken from dogs that consumed baits the previous day.

**Test 2** This protocol seeks to determine whether dog owners given baits at a central site(s) will feed such baits to their dogs and if dogs would have been vaccinated as subsequently estimated by the presence of a systemic biomarker in their blood sera. The protocol also includes acquisition of data on dog density, dog ownership, numbers of baits required, and cost/benefit information.

**Test 3** This field trial involves determining the efficacy of vaccinating owned dogs by door to door bait distribution. It also provides for obtaining an estimate of the owned dog population by using a "capture-mark-recapture" technique based upon the percent of dogs subsequently observed that are marked with collars or dye markers.

**Test 4** This protocol is intended to assess bait distribution when baits are placed and left overnight for unconfined free-ranging dogs. When conducted concurrently with test 3 above, it seeks to determine the costs and percentages of both owned dogs and all dogs reached by baits using both house to house and overnight bait distribution.

This paper reviews the present status of dog vaccine baits, suggests materials for improving baits, discusses techniques for determining bait preferences and outlines WHO recommendations for assessing bait distribution strategies. In conclusion, interest in the oral rabies vaccination of dogs is recent and efforts to develop appropriate baits and delivery techniques have been modest. More work will be required to determine its role as a rabies control method.

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