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Improving Rabies Vaccine Baits

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ABSTRACT: The U.S. Department of Agriculture distributes more than 10 million tetracycline-containing rabies vaccine baits to control the spread of wildlife-vectored rabies to humans, pets, and livestock. To estimate the percent of target species consuming the baits, raccoons were collected in baited areas and teeth were analyzed for the presence of the tetracycline biomarker. Several incidents of low biomarker detection rates prompted an investigation of the stability of the biomarker in the baits. These studies indicated that a portion of the tetracycline was converted to epitetracycline. Additionally, significant quantities of both compounds were trapped in the polymer which is homogeneously distributed throughout the bait. This situation is likely responsible for low biomarker detection rates. To alleviate this problem, we developed an alternative bait matrix which permits increased stability and bioavailability of the tetracycline biomarker. This new bait matrix increased the availability of the tetracycline marker from 25.2 to 87.3% and decreased epitetracycline formation from 12.4 to 3.6%.

KEY WORDS: baits, biomarker, *Procyon lotor*, rabies, raccoon, tetracycline, vaccine

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INTRODUCTION

In an effort to mitigate the western migration of terrestrial wildlife-vectored rabies in the United States, the U.S. Department of Agriculture Wildlife Services Program (USDA WS) has distributed more than 40 million vaccine baits, including 10.5 million rabies vaccine baits in 2003 (Kuehn 2003, Anonymous 2003). These baits consist of a sachet containing oral rabies vaccine surrounded by a fishmeal polymer. To permit a cost-effective means of monitoring bait consumption by target and non-target species, the polymer was formulated to contain 1% tetracycline (Figure 1) as a biomarker. As an indicator of bait consumption, USDA employees remove the first premolar in captured raccoons (*Procyon lotor*) for subsequent analysis of tetracycline deposits. In some instances, rabies virus neutralizing antibody levels in the blood of captured wildlife are also monitored. Following a recent rabies vaccine bait application in Ohio, antibody analyses indicated that 40% of raccoons in the bait zone were antibody positive, yet only 10% of the population was positive for the tetracycline biomarker (D. Slate, pers. commun.). These findings led us to investigate the biomarker efficiency of the tetracycline in the rabies vaccine baits. Our studies indicated that about 40% of the tetracycline contained in the USDA rabies baits was unavailable for absorption due to conversion of tetracycline to epitetracycline and entrapment in the plastic containing bait matrix (Johnston *et al.* 2005). To overcome this obstacle, we developed a bait matrix that contains a relatively low melting point polymer (to limit conversion of tetracycline to epitetracycline) and that disintegrates in stomach acid. Additionally, we included sodium bicarbonate to facilitate the disintegration of the bait matrix in stomach acid.

MATERIALS AND METHODS

HCl was obtained from Fisher Scientific (Fair Lawn, NJ). Menhaden fish meal (Bait-Tek, Beaumont, TX), fish meal oil (Catch n' Bait, Brooksville, FL), Eudragit

EPO polymer (Rohm and Hass, Philadelphia, PA), tetracycline (Aldrich Chemical, St. Louis, MO), and sodium bicarbonate (Aldrich) were mixed in a Hobart mixer until homogeneous (Table 1). Approximately 20 grams of this mixture was transferred to a stainless steel cylinder which was heated to 60°C. The cylinder contents were pressurized to 24,000 psi per for 15 minutes. Upon cooling, the circular contents were broken into small pieces (ca. 3 cm in diameter) and placed in a Petri dish containing either pH 7 water or pH 2 0.1N HCl. This process was also repeated using ground USDA rabies bait matrix.

The Petri dishes were slowly shaken on a horizontal mixer for 120 minutes. The solutions were filtered, diluted 1:10 with water, and analyzed by HPLC to permit quantification of extracted tetracycline and epitetracycline (Table 2) (Johnston *et al.* 2005).

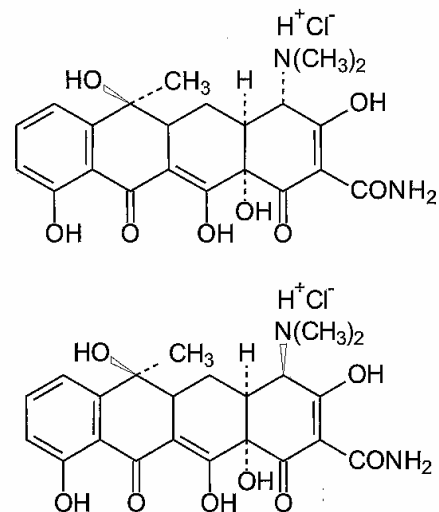


Figure 1. Tetracycline (above); epitetracycline (below).

Table 1. Recipe for new rabies vaccine bait matrix.

Ingredient	Percent
fish meal	75
fish oil	5
polymer	15
tetracycline	1
sodium bicarbonate	4

Table 2. HPLC conditions.

Mobile phase	0.05N H ₃ PO ₄ :ACN (80:20)
Flow rate	0.5 mL/min
Oven temperature	Ambient
HPLC column	Polymer Laboratories (Amherst, MA), PLRP-S 150 x 4.6 mm i.d., 5 µm particle size
Injection volume	10 µL
Absorbance	365 nm
HPLC run time	10 minutes

Table 3. Tetracycline recovery from baits.

	Mean Recovery			
	Tetracycline*		Epitetra-cycline*	
	pH 7	pH 2	pH 7	pH 2
Current Bait	<1%	25.2%	<1%	12.4%
New Bait	<1%	87.3%	<1%	3.6%

*CV < 0.1%

RESULTS

Less than 1% of the tetracyclines in both bait matrices were extracted into the pH 7 water. However, the pH 2 solution extracted an average of 37.6% or 90.9% of the tetracyclines (tetracycline + epitetracycline) originally contained in the current USDA or the new bait matrix, respectively. Additionally, the HPLC analyses indicated that 33% (epitetracycline / total tetracyclines) of the tetracyclines in the current USDA bait had been converted to epitetracycline (Figure 1) compared to 4% for the new bait formulation (Table 3).

DISCUSSION

Our previous research showed that the heat extrusion process used to manufacture the current USDA rabies vaccine baits converts approximately 1/3 of the tetracycline to epitetracycline, a less efficient biomarker. Furthermore, the plastic matrix used in the current USDA

baits is nearly insoluble in stomach acid, which appears to limit the absorption of the tetracycline and epitetracycline. In an attempt to illustrate how reformulation of the current bait matrix could significantly increase the bioavailability of the tetracycline, we prepared a bait matrix with a lower melting point polymer to minimize the heat-induced conversion of tetracycline to epitetracycline. Additionally, the plastic polymer contains a tertiary amine that is converted to an ionic quaternary amine in stomach acid, which increases the solubility of the matrix (Figure 2). Finally, in the presence of stomach acid, the release of carbon dioxide from the sodium bicarbonate facilitated the physical disintegration of the bait. This increased the surface area of the bait matrix, which led to increase solubilization of the tetracycline.

When suspended in pH 7 water, both matrices released less than 1% tetracyclines. This suggests that both bait matrices afford similar protection against tetracycline leaching under environmental conditions. However, in simulated stomach acid, only about 1/3 of the tetracyclines were liberated from the current USDA bait, as compared to liberation of more than 90% of the tetracyclines from the new bait matrix. Additionally, since the new matrix can be formulated at lower temperatures, only a minimal quantity of tetracycline was converted to epitetracycline. We believe this experiment clearly indicates the potential benefit of rabies vaccine bait reformulation for the USDA rabies program.

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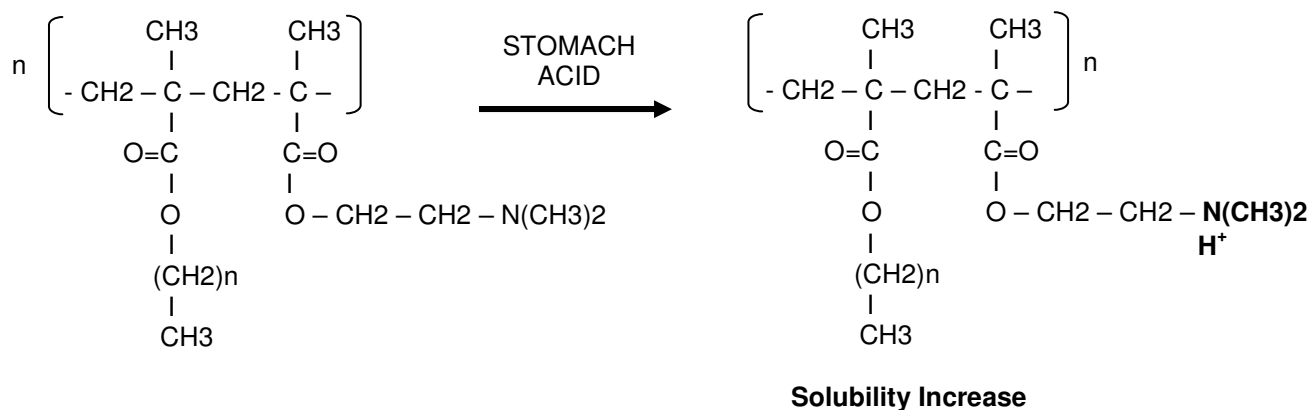


Figure 2. Mechanism for increased solubility of bait matrix in stomach acid.