Iophenoxic Acid and Rhodamine B as Biomarkers of Bovine Tuberculosis Vaccine Bait Uptake by White-tailed Deer

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ABSTRACT: Bovine tuberculosis (bTB), caused by the bacteria *Mycobacterium bovis*, exists in free-ranging white-tailed deer in portions of northeastern Lower Michigan where deer herds serve as reservoirs and sources for reinfection of livestock herds. Density reduction and vaccination of reservoir host populations could be used together to reduce prevalence or eliminate the disease. Voluntary oral uptake of vaccine by deer is the most feasible mode of delivery. High probability of eliminating bTB would depend, in part, on a high proportion of deer being vaccinated. Chemical biomarkers could be used to estimate the proportion of a deer population consuming baits. Three analogs of iophenoxic acid were evaluated relative to their pharmacokinetic profile in blood-serum, and presence of rhodamine B was evaluated in facial whiskers using captive white-tailed deer.

KEY WORDS: biomarkers, blood serum, bovine tuberculosis, disease, iophenoxic acid, *Odocoileus virginianus*, rhodamine B, vaccine delivery, white-tailed deer

INTRODUCTION

Domestic cervid and livestock producers, veterinarians, wildlife managers, and human health organizations around the world are concerned by the occurrence of bovine tuberculosis (bTB), caused by the bacterium *Mycobacterium bovis* (Thoen et al. 2009). White-tailed deer (*Odocoileus virginianus*) in the USA can act as reservoirs of bTB and provide a source for infection of livestock (Schmitt et al. 1997, O'Brien et al. 2002). Population reduction and vaccination of reservoir host populations could be used together to reduce prevalence or eliminate the disease. However, more information is needed on delivery methods for distributing vaccine baits to free-ranging white-tailed deer. Relative preference among alternative baits and proportion of the deer population consuming baits are important parameters to estimate before attempting vaccine delivery. Chemical biomarkers have previously been used for similar purposes (Fletcher et al. 1990, Marks and Bloomfield 1999, Fleming et al. 2000, Southey et al. 2001, Cagnacci et al. 2007).

Many biomarking chemicals have been evaluated for use in wild and feral animals for, among other purposes, detecting whether individuals have consumed baits (Savarie et al. 1992). These can be long-term systemic markers, like tetracycline which accumulates in bones and teeth and is detected by fluorescence under ultraviolet (UV) light (Van Brackle et al. 1994, Matson and Kerr 1998); rhodamine B (RB) which accumulates in keratinous tissues (e.g., claws, hair, and vibrissae or whiskers) and is also detected by UV fluorescence (Fisher 1999); and iophenoxic acid (IPA), which binds with blood proteins where presence in serum is indirectly inferred by detection of elevated serum iodine (Knowlton et al. 1988, Hadidian et al. 1989) or, more recently, by chromatographic techniques for direct quantitation of IPA in serum (Jones 1994, Wiles and Campbell 2006).

Tetracycline is best used during growth periods where maximal deposition of bone and tooth material can assimilate the marker in visible quantities (Matson's Laboratory 2011). As we envision deploying baits during winter, a period of reduced dietary intake and tissue growth for white-tailed deer, tetracycline would not be suitable for our application. Requirements for destructive sampling of tissues (euthanasia of animals or removal of teeth) and controversy over releasing tetracycline into the environment provide incentive to identify alternative biomarkers (Fry et al. 2010).

Rhodamine B has proven effective for determining whether individual animals have consumed baits or not (i.e., qualitative marker; Fisher 1999, Cagnacci et al. 2006, Cagnacci et al. 2007, Fry et al. 2009, Fry et al. 2010, Ballesteros et al. 2011). It has been used on a variety of species including rodents (Lindsey 1983, Jacob et al. 2002, Rahelinirina et al. 2009), mesocarnivores (Johns and Pan 1981, Knowlton et al. 1988, Spurr 2002, Cagnassi et al. 2007, Fry et al. 2010), lagomorphs (Evans and Griffith 1973), feral pigs (Flemming et al. 2000), and white-tailed deer (Webb et al. 2000). Although Webb et al. (2000) tested RB as a fecal biomarker in white-tailed deer, fecal marking lasts only a few days and they did not evaluate presence and longevity of marks in whiskers, which has not been documented for deer.

Iophenoxic acid (-ethyl-3-hydroxy-2,4,6-triiodohydrocinnamic acid, Et-IPA) is a relatively long-lasting qualitative biomarker of blood serum that also has potential for estimating quantity of bait consumed (quantitative marker; Eason and Batcheler 1991, Massei et al. 2009). Several analogues of Et-IPA have been developed presenting the radicals butyl (Bt-IPA), methyl (Mt-IPA), pentyl (Pt-IPA), and propyl (Pr-IPA), rather than ethyl. These analogues may potentially facilitate simultaneous testing of multiple bait types or identifying temporal patterns in bait uptake (Jones et al. 1997, Ballesteros et al.

2010). The useful duration of marking is typically different among IPAs (Jones et al. 1997, Massei et al. 2009, Ballesteros et al. 2010) and is further dependent on species of animal (Sweetapple and Nugent 1998) and sensitivity of analytical method (Jones 1994).

We evaluated the pharmacokinetic profile of Et-IPA, Bt-IPA, and Pr-IPA in blood serum for 6 months after dosing captive white-tailed deer. Although White et al. (1995) evaluated Et-IPA as a serum marker in white-tailed deer, they measured total serum iodine, where iodine attributable to IPA is confounded with iodine from dietary sources. For use in field applications, this variable requires independent estimation of background concentrations of iodine in a target population and substantially reduces sensitivity of detection and duration of the reliable period for classifying marked individuals. Newer, more sensitive analytical methods have been developed to directly and simultaneously measure concentrations of multiple IPAs present in serum, independent of other serum iodine sources (Jones 1994, Purdey et al. 2003, Wiles and Campbell 2006, Ballesteros et al. 2010). Our study will quantify the dose-response-time relationship for each IPA using advanced analytical techniques with extended limits of detection and quantification of serum concentration. Results will indicate the useful window of marking for each IPA, the time required for each to fall below detection limits, and error levels associated with using each as a quantitative indicator of ingested dose.

METHODS

Captive white-tailed deer (Deer Research Center, Pennsylvania State University) were administered IPAs and RB (based on body mass, BM) orally to achieve a prescribed dose (mg/kg BM). Both biomarkers were administered in gelatin capsules delivered postlingually into the esophagus using a balling gun. Deer were periodically monitored during the first 2 days after dosing for evidence of adverse reactions.

Twenty-seven deer were allocated to 5 treatment groups representing a different IPA dose: 0, 2.5, 5, 25, and 50 mg/kg BM. Each deer received equal amounts (e.g., 5 mg/kg) of each of the 3 IPA types, for each treatment group. White et al. (1995) suggested 5 mg/kg as an appropriate single dose for biomarking white-tailed deer. We established additional dose levels corresponding to 0.5 times, 5 times, and 10 times the level of a single dose, to evaluate potential for quantifying bait uptake based on post-baiting observed serum IPA concentrations and time after baiting.

The control group consisted of 3 deer: 1 mature doe, 1 yearling doe, and 1 fawn buck, which were dosed with 2 placebo capsules containing cornstarch. The remaining groups consisted of 2 mature does, 2 yearling does, 1 fawn doe, and 1 fawn buck. Animals within age-sex classes were randomized to IPA treatment groups. One randomly selected deer from each age-sex class within each IPA treatment group also received RB at 15 mg/kg BM.

Blood was drawn by jugular venipuncture into tubes containing EDTA (Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ) from each deer immediately before dosing, and then 1 d after dosing; 1, 3, 5, 7, 9, 11, and 13 weeks after dosing; and 4, 5, 6, and 7 months after dosing. Two to 3 tubes of approximately 3 mL each were collected at each sampling. Serum was separated by centrifugation within 2 hours of collection and stored at -40°C. Serum was analyzed to estimate concentration of individual IPAs using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) in multiple-reaction monitoring (MRM) mode, using negative mode electrospray ionization.

Whiskers for RB sampling were collected at 1, 3, and 5 weeks and at 7 months using a forceps. On each occasion, 3 maxillary whiskers were extracted from each side of the rostrum. After the initial sampling occasion, short, potentially "regrowth" whiskers were avoided. Fluorescent microscopy was used to detect marking bands on whiskers indicating deposition of RB (Fisher 1999, Fry et al. 2009).

RESULTS and DISCUSSION

Although analyses are currently incomplete, preliminary results indicate that the 3 IPA analogs differ in their pharmacokinetic profile in serum, with Et-IPA persisting the longest and Bt-IPA having the shortest persistence. Preliminary results from RB analysis indicate a high proportion of maxillary whiskers were marked out to 5 weeks after dosing. All types of IPA were below detection limits (150-170 ppt, depending on IPA type) by 26 weeks after dosing, suggesting deer ingesting IPA during vaccine bait deployments anticipated to occur in January and February would be essentially clear of IPA by hunting seasons beginning in September. Similarly, deer would also be clear of RB before hunting seasons. Thus, IPA and RB biomarkers, when used as anticipated, would likely prove useful for estimating exposure to vaccine without posing risk of entering the human food supply.

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