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Long-lasting Systemic Bait Markers for Eurasian Badgers

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ABSTRACT: This study was carried out to assess whether Rhodamine B, ethyl-iophenoxic acid (EtIPA), and propyl-iophenoxic acid (PrIPA) can be used as long-lasting systemic bait markers for free-living badgers (*Meles meles*). Between June and November 2003, these chemicals were incorporated into bait distributed around badger setts. Serum, hair, and whiskers from individually marked badgers were collected in the following 4 to 24 wk. Rhodamine B was detectable as fluorescent bands up to 24 wk after ingestion of the bait. Individual badgers were found positive for EtIPA and PrIPA up to 20 wk and 18 wk after exposure, respectively. This study indicates that Rhodamine B, PrIPA, and EtIPA could be used as long-lasting markers for badgers.

Key words: Bait markers, European badger, iophenoxic acid, *Meles meles*, Rhodamine B, vaccination.

Chemical bait markers are compounds incorporated in bait to enable identification of individuals that have consumed the bait and have been used to monitor bait uptake for orally delivered vaccines and toxicants (Morgan, 1982; Trewhella et al., 1991). The Eurasian badger (*Meles meles*) is one of the main wildlife reservoirs for the transmission of bovine tuberculosis to cattle. Recently, vaccination in combination with fertility control has been suggested as a long-term strategy to limit the spread of tuberculosis between badgers and to reduce transmission to cattle (Smith and Cheeseman, 2002). Bait markers are thus required to determine the proportion of badgers ingesting bait-delivered vaccines or fertility control agents. Because large-scale vaccination or fertility control campaigns are likely to rely on late captures to determine the proportion of individuals that have ingested bait, persistent bait markers would be a preferable option to short-term markers. Furthermore, if multiple markers were available, they could provide information on temporal or spatial patterns of bait consumption. The seromarker iophenoxic acid and the marker of keratinous tissues Rhodamine B might meet these requirements. Ethyl-iophenoxic acid (EtIPA) has been used successfully as a long-term bait marker in many wild carnivores, including badgers (Saunders et al., 1993; Southey et al., 2001; de Leeuw et al., in press). The persistence of EtIPA and Rhodamine B is highly variable between species (Fisher, 1999; Spurr, 2002). For instance, EtIPA can be detected for up to 18 wk in badgers (Southey et al., 2001) and 8 wk in foxes *Vulpes vulpes* (Jones et al., 1997), but only for 2 wk in stoats (Spurr, 2002). Propyl-iophenoxic acid (PrIPA), so far tested only on foxes and badgers, can be detected for 7–8 wk (Jones et al., 1997; de Leeuw et al., in press). Rhodamine B bands were detected for up to 12 wk in whiskers of house mice *Mus musculus* (Jacob et al., 2002) and up to 28 wk after exposure in hair of mountain beavers *Aplodontia rufa* (Lindsey, 1983). In free-living badgers, Rhodamine B was tested as a short-term (5 days) bait marker of hair (Southey et al., 2002) but its long-term persistence was not investigated. In this study, we aimed to 1) compare the persistence of EtIPA and PrIPA in free-living badgers and 2) determine the persistence of Rhodamine B in whiskers and hair of different body regions of free-living badgers.

The study was conducted in 2003 at Woodchester Park Badger Research Station (UK; 51°43′N, 2°16′E) as part of a field experiment (Cagnacci et al., 2005) that used three types of bait (prunes, minced meat, and cereals mixed with molasses). The three markers used were Rhodamine B (Sigma-Aldrich Co., Poole, UK), EtIPA (α-ethyl-2-hydroxy-2,4,6-triiodebenzenepropanoic acid, Sigma-Aldrich), and its propyl analogue (PrIPA).

The Pr-IPA was custom-synthesized (Jones et al., 1997). Rhodamine B–treated bait was prepared by mixing 100 mg of Rhodamine B to each bait. This dose was equivalent to approximately 11–12 mg/kg of badger body weight. The IPA-treated bait was prepared by adding 40 mg per bait of either EtIPA or PrIPA to 1 M NaOH and then mixing the resulting salt to the bait. This dose was equivalent to approximately 4–5 mg/kg of badger body weight. Bait never contained more than one marker at a time. Each bait type was used with each marker, producing nine bait-marker combinations. The bait was distributed in the area surrounding the main active sett (as in Delahay et al., 2000) in June, July, and October 2003. Badgers were offered bait treated with EtIPA (10 July, 13–15 October), PrIPA (23 July, 3–12 October), and Rhodamine B (12 June, 9 July, and 1 October), ensuring that different social groups were targeted in June, July, and October. Samples of serum and hair were obtained from badgers trapped at different intervals after exposure to Rhodamine B and IPA. To test for the occurrence of false positives, additional serum and hair samples were collected from badgers (controls) captured in areas that were not contiguous with those exposed to the bait containing markers. Each animal was identified by a unique tattoo on the belly. Guard hairs, including the follicle, were collected from flanks, nape, and rump. Because whiskers act as tactile sense organs, a single whisker per badger was cut at the base near the animal's skin. Hair and whiskers were viewed at 10× magnification with an ultraviolet (UV) fluorescence microscope. Twenty hairs from each body region were analyzed for each badger. Hair samples were recorded as positive when a brilliant orange fluorescent band was observed in the follicle or along the hair (Fisher, 1999). The distance from bulb to band was measured to the brightest point of the band. The concentration of both IPA analogues in the serum samples was determined as described in Jones (1994) and Jones et al. (1997).

Samples from seven badgers were positive for Rhodamine B (Fig. 1). All samples from the control group were negative. Whiskers from six of seven positive badgers showed a clear fluorescent band. Nape hairs showed fluorescent bands in five of seven positive badgers, whereas fluorescent bands in flanks and rump hairs were found in all positive badgers (Fig. 1). Fluorescent bands were detected in hairs sampled up to 24 wk after exposure (Fig. 1), as well as after 22, 10, 8, and 4 wk. The position of the fluorescent band in relation to the hair length depended both on the time of exposure and on the sampling interval. For three badgers (40G, T47, D73; Fig. 1) exposed to Rhodamine B in early summer and sampled 10 wk later, fluorescence was found only in the bulbs of the hair, whereas whiskers had a distinct band. Samples from one animal (D73) were all negative; however, when the same individual was resampled in autumn, fluorescent bands were observed close to the tips of the hairs. Hairs of badgers exposed to Rhodamine B in early autumn and sampled 8 wk later showed fluorescent bands.

Because Rhodamine B is incorporated in keratinous tissues such as hair and whiskers, its persistence depends on the molting cycle of each species. Badgers molt once a year from July through December when hair follicles are in the growth phase. In January, when the molt is complete, the follicles enter a resting stage, and from February to July no hair is lost or grown (Maurel et al., 1986). Our results with Rhodamine B were consistent with the badgers' molting cycle. Hairs of individuals exposed early in the molting season (June) and sampled in autumn showed fluorescent bands. Hairs of individuals exposed in early autumn and sampled a few weeks later showed bands close to the bulb because hairs were almost fully grown when the Rhodamine

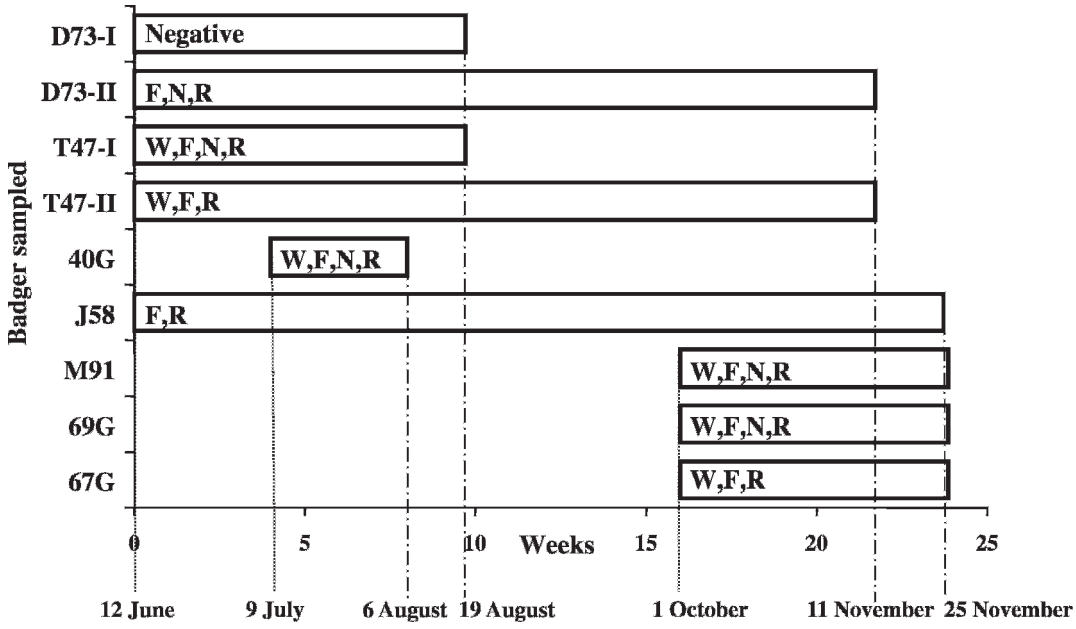

FIGURE 1. Persistence of Rhodamine B bands in the hair of seven badgers captured at different intervals from ingestion of bait treated with Rhodamine B. Dotted lines indicate date of exposure of each individual to Rhodamine B; striped lines indicate date of capture and sampling. D73 and T47 were sampled twice. The letters in the bars indicate the types of hair of each individual that were positive by ultraviolet fluorescence microscopy analysis. W = whisker; F = hair from flanks; N = hair from nape; R = hair from rump; Negative = no fluorescent bands detected.

B was fixed. The results from badger D73, found negative to Rhodamine B 10 wk after exposure but positive 22 wk later, suggested that Rhodamine B was incorporated into the resting cells of the hair follicle. Fluorescent bands became visible when the follicle cells returned to the active phase. In badgers, the use of Rhodamine B as a systemic marker of hair might therefore not be limited to times of the year when active coat growth occurs. However, to avoid recording false negatives, sampling for Rhodamine B should occur during the active coat growth phase from July to December.

Eighteen badgers were found positive to EtIPA up to 20 wk after exposure, and 10 badgers were found positive to PrIPA up to 18 wk after exposure (Table 1). All serum samples from control groups were negative for both EtIPA and PrIPA. For badgers potentially exposed to IPA-marked bait, it was impossible to establish the proportion of false negatives (i.e., badgers that had ingested IPA-marked bait but had eliminated all the IPA). However, the results of this study were consistent with those obtained by Southey et al. (2001) showing that, in captive badgers, EtIPA can persist for at least 18 wk after ingestion. This study confirmed these results with wild badgers and suggested that both EtIPA and PrIPA can be potentially used as long-term indicators of bait uptake. Future studies with individually marked badgers trapped at regular intervals might quantify the proportion of false negatives over time.

The results of this study also indicated that Rhodamine B is a safe, potentially long-term marker for identifying badgers that have ingested bait. Fisher (1999) indicate that at the doses commonly used in wildlife management, Rhodamine B does not pose risks of carcinogenicity or mutagenicity. Advantages of Rhodamine B

TABLE 1. Concentration (Conc.) of ethyl-iophenoxic acid (EtIPA) and propyl-iophenoxic acid (PrIPA) in the sera of badgers sampled at different intervals from ingestion of bait containing one of the two compounds.

			EtIPA				PrIPA			
Badger ID	Sex	Age[a]	Date of exposure[b]	Date of sampling[b]	Conc. (μg/ml serum)	Weeks after exposure	Date of exposure[b]	Date of sampling[b]	Conc. (μg/ml serum)	Weeks after exposure
40G	M	C	10 July	5 August	14.8	4	23 July	5 August	—	—
Q08	F	A	10 July	5 August	0.5	4	23 July	5 August	—	—
D073	M	A	10 July	18 August	1.0	6	23 July	18 August	—	—
I24	F	A	10 July	18 August	0.7	6	23 July	18 August	—	—
U68	F	A	10 July	18 August	0.8	6	23 July	18 August	—	—
M64	F	A	10 July	2 September	4.2	8	23 July	2 September	6.4	6
M109	F	A	10 July	2 September	4.0	8	23 July	2 September	0.7	6
Q94	M	A	10 July	2 September	8.8	8	23 July	2 September	—	—
22G	M	C	10 July	2 September	3.4	8	23 July	2 September	22.4	6
J58	M	A	10 July	24 November	0.8	20	23 July	24 November	1.8	18
27G	M	C	10 July	2 September	19.5	8	23 July	2 September	—	—
32G	F	C	10 July	2 September	26.5	8	23 July	2 September	—	—
U49	M	A	10 July	18 August	0.5	6	23 July	18 August	—	—
U57	F	A	10 July	18 August	0.8	6	23 July	18 August	—	—
U67	F	A	10 July	18 August	0.8	6	23 July	18 August	—	—
65G	F	C	13–15 October	24 November	—	—	3–12 October	24 November	3.4	6
66G	F	A	13–15 October	24 November	8.4	6	3–12 October	24 November	12.7	6
67G	F	C	13–15 October	24 November	—	—	3–12 October	24 November	7.5	6
72G	F	C	13–15 October	24 November	12.0	6	3–12 October	24 November	12	6
M91	F	A	13–15 October	24 November	—	—	3–12 October	24 November	0.5	6
69G	M	C	13–15 October	24 November	2.6	6	3–12 October	24 November	1.1	6

[a] C = cub; A = adult.

[b] All dates refer to year 2003.

compared with PrIPA and EtIPA, are cost effectiveness, in terms of market price of the compound and identification assay, and detection, which for Rhodamine B is less invasive and does not require anesthesia. This is particularly relevant for large-scale baiting campaigns, when a large quantity of bait and bait markers would be used (Southey et al., 2002).

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