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Testing of a palatable bait and compatible vaccine carrier for the oral vaccination of European badgers (*Meles meles*) against tuberculosis

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ABSTRACT

The oral vaccination of wild badgers (*Meles meles*) with live Bacillus Calmette–Guérin (BCG) is one of the tools being considered for the control of bovine tuberculosis (caused by *Mycobacterium bovis*) in the UK. The design of a product for oral vaccination requires that numerous, and often competing, conditions are met. These include the need for a highly palatable, but physically stable bait that will meet regulatory requirements, and one which is also compatible with the vaccine formulation; in this case live BCG. In collaboration with two commercial bait companies we have developed a highly attractive and palatable bait recipe designed specifically for European badgers (*Meles meles*) that meets these requirements. The palatability of different batches of bait was evaluated against a standardised palatable control bait using captive badgers. The physical properties of the bait are described e.g. firmness and colour. The microbial load in the bait was assessed against European and US Pharmacopoeias. The bait was combined with an edible vaccine carrier made of hydrogenated peanut oil in which BCG vaccine was stable during bait manufacture and cold storage, demonstrating <0.5 $\log_{10}$ reduction in titre after 117 weeks' storage at −20 °C. BCG stability in bait was also evaluated at +4 °C and under simulated environmental conditions (20 °C, 98% Relative Humidity; RH). Finally, iophenoxic acid biomarkers were utilised as a surrogate for the BCG vaccine, to test variants of the vaccine-bait design for their ability to deliver biomarker to the gastrointestinal tract of individual animals. These data provide the first detailed description of a bait-vaccine delivery system developed specifically for the oral vaccination of badgers against *Mycobacterium bovis* using live BCG.

1. Introduction

The package of control measures aimed at the eradication of bovine tuberculosis (TB) in England and Wales includes the development of an oral vaccine for badgers (*Meles meles*) against the causative agent, *Mycobacterium bovis* [1]. The first injectable vaccine for TB in badgers, BadgerBCG, was licensed in 2010 and has been used in specific areas of England and Wales since then [2,3]. The beneficial effects of the injectable vaccine have been demonstrated in terms of reducing disease severity and progression in captive badgers and reduced serological evidence of infection in wild badgers [4]. The major limitation of BadgerBCG is the need to trap badgers to inject the vaccine. A cost-effective BCG-based oral vaccine could achieve wider coverage, overcoming

Abbreviations: HPO, hydrogenated (hardened) peanut oil; QC, quality control; PT, paste-bait; EP, European pharmacopoeia; USP, United States pharmacopoeia; IPA, iophenoxic acid; TAMC, total aerobic microbial count; TYMC, total yeast and mould count.

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some of the financial and logistical issues associated with the widespread deployment of BadgerBCG [5].

Oral vaccines against TB are in development for a number of wildlife species besides badgers [6–9]. In all cases, the development and delivery of a licensed oral vaccine product to the field faces many challenges, including effective delivery of the vaccine by consumption, vaccine stability, and environmental safety [10]. These are best exemplified by the comprehensive programme of research and development of the oral rabies vaccine for foxes (*Vulpes vulpes*) in Europe [11], the end product being a 'tailor-made' species-specific bait-vaccine product that can be manufactured and deployed at scale.

Oral vaccine delivery mechanisms developed for one species are not necessarily appropriate for another, even if the product is palatable. For example, a bait-vaccine carrier developed for wild boar (*Sus scrofa*) was also attractive to badgers in Spain, but of 150 baits consumed by badgers, 87% had the vaccine carriers (plastic capsules) rejected and of these, 99% were separated from the bait intact with the payload of water still inside [12].

Here we present the first detailed description of a vaccine delivery system developed specifically for the oral vaccination of badgers with live BCG. Numerous baits and possible vaccine carriers were trialed with both captive and wild badgers. Captive animals were used to screen large numbers of different products of which some were selected for field-testing; only selected data are presented here. The selection of the best bait was dependent on many factors including the potential for ease of manufacture at relatively low cost, as well as the results of associated field studies. The data we present are crucial for the on-going development and eventual licensing of an oral vaccine product for badgers, including: (a) identification and description of important physical characteristics of the bait for potential future quality control (QC) purposes for large-scale manufacture; (b) design of a bait with a compatible and palatable vaccine carrier; (c) evidence that the bait-vaccine carrier design can deliver biomarker to the GIT of badgers as a surrogate for BCG; and (d) evidence that the BCG vaccine remains viable in the delivery system through laboratory production processes, cold-storage and simulated field conditions.

2. Materials & methods

2.1. Animals

Badgers were either brought in from the wild from TB-free areas under licence, or born in captivity. Wild-caught animals were demonstrated to be free of TB on the basis of IFNγ and clinical sample culture testing and housed in groups (two to five animals per pen) in open-air pens with artificial setts, as described elsewhere [13]. Animals were fed a mixture of commercial dog food, peanuts and occasionally fruit and specified pathogen-free eggs. Each pen was equipped with a motion-sensitive infra-red CCTV camera (Secom Security Systems PLC., Kenley, UK). Groups of two to five penned animals were used in palatability and bait design tests as animals could not be repeatedly housed individually for animal welfare reasons; animals were individually caged for a single night for the biomarker study only. The work was carried out under licences (PL 70/6864 and PL 70/7878) from the UK Home Office under the Animal (Scientific Procedures) Act 1986 and approved by the Animal and Plant Health Agency (APHA) Local Ethical Review Panel.

2.2. QC of the bait components

The bait (referred to as either 'PT' or 'paste bait'), is based on a proprietary recipe and was developed with Pest-Tech Ltd. (Leeston, New Zealand) and Connovation Ltd. (Manukau, New Zealand). The paste is free of anti-microbial preservatives, genetically-modified organisms or animal-derived products. Two physical attributes, namely firmness and colour, were assessed for batches of paste bait post-production. Firmness measurements (kgf) were obtained using a calibrated fruit pressure tester (FT011 with 8 mm tip; ACE Supplies Industrial Ltd., Staplehurst, UK) applied to a minimum of three bait portions ($\sim$11 g) from each batch. Colour was visually assessed by comparison with a colour chart [14] and the closest match recorded for each batch.

Between one and three samples of paste bait from each batch were submitted for microbiological testing (Wickham Laboratories Ltd., Gosport, UK) as soon as possible after manufacture to assess microbial burden against the European pharmacopoeia (EP) and US pharmacopoeia (USP) specifications for 'Non-aqueous preparations for oral use': (a) total aerobic microbial count (TAMC) and total yeast and mould count (TYMC) with limits of $\leqslant 2 \times 10^3$ and $\leqslant 2 \times 10^2$ CFU g^{-1} of bait, respectively; and (b) the absence of *Escherichia coli* in 1 g of material. Where more than one sample was tested per batch of bait, if any one sample exceeded any of the EP or USP specifications, it was considered to have failed QC. Three batches of the vaccine-carrier material, a solid, edible vegetable lipid (HPO, hardened [hydrogenated] peanut oil; Ph. Eur., Sigma-Aldrich Company Ltd., Gillingham, UK) were also submitted for microbiological testing against EP and USP criteria.

Palatability testing of the paste bait was carried out between April and October, in both 2013 and 2014, in order to avoid the winter months when badgers exhibit reduced activity [15] and to correspond to when oral bait vaccine would most likely be deployed in the field. In each test (one test per batch of bait) between six and eight groups of animals were each presented with bait contained in five litre plastic tubs (two white and two grey), which were placed in a Latin square arrangement in each pen (tubs approx. 1 m from each other) in the late afternoon before the animals emerged from their setts. Two tubs contained 400 ± 1 g of a batch of paste bait each and two tubs contained 400 ± 1 g of control bait each, comprising a mix of peanuts and golden syrup (ratio 8:1), known to be highly palatable to badgers [16]. Tests were run overnight and normal feed was either withheld for the entire night or given to a group after they had consumed some, but not all, of either bait type; water was provided *ad libitum*. Limited consumption of bait by a group of animals (i.e. <20 g of one or both bait types, as recommended by the manufacturer) was not considered to be representative of a definitive preference and could result in incorrect palatability calculations. Therefore groups which consumed <20 g of material were not included in the palatability calculations. Palatability (%) was calculated for each group of animals by dividing the weight of test bait consumed by the total quantity of bait (test and control) consumed by the group. The final palatability value for a batch of bait was calculated by taking an average across all groups. The peanut and syrup control provided a benchmark for palatability, as a minimum standard for a palatable bait. Therefore any test material was required to be at least as palatable as the control i.e. have a palatability of $\geqslant$50%. However, a more stringent palatability threshold of 65% was set for this work to allow for the greater variability introduced when using a small number of groups for testing; ideally palatability tests would utilise large numbers of individually caged animals (R. Henderson, Pest-Tech Ltd., personal communication).

2.3. Bait design tests: bait consumption

Variants of the PT-HPO bait design (Fig. 1) were evaluated in two tests designed to investigate whether altering the PT:HPO ratio and varying the surface area of the exposed PT (to enhance odour release) affected bait disappearance and/or preference.