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Behaviour of European badgers and non-target species towards candidate baits for oral delivery of a tuberculosis vaccine

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ABSTRACT

In the UK and the Republic of Ireland, the European badger (*Meles meles*) is a maintenance host for *Mycobacterium bovis*, and may transmit the infection to cattle causing bovine tuberculosis (TB). Vaccination of badgers using an injectable Bacillus Calmette-Guerin (BCG) vaccine is undertaken in some areas of the UK with the intention of interrupting this transmission, and vaccination research is underway in Ireland. An oral badger TB vaccine is also under development. We investigated the behaviour of badgers and non-target wildlife species towards three candidate baits being considered for delivering BCG to badgers orally. Bait preference was investigated by recording removal rates of baits and through the use of video surveillance at 16 badger setts. We found high variation in rates of bait removal by badgers among setts but no significant differences in removal rates among bait types or in preference behaviour from video footage. Variation in bait removal among setts correlated with the number of nights on which badgers were seen at the sett, with most baits being removed where badgers were seen on >50% of nights during the ten-day study period. Relatively few baits were removed at setts with low levels of recorded badger activity. Monitoring badger activity prior to bait deployment may therefore be useful in increasing bait uptake and vaccine coverage. Bait removal by badgers increased over the ten-day study period, suggesting initial neophobic behaviour at some setts and that a period of 'pre-feeding' may be required prior to vaccine deployment. Our results indicate that all three candidate baits are attractive to badgers. Removal of baits by non-target wildlife species was generally low, but varied among bait types, with smaller baits in packaging less likely to be removed. Enclosing baits in packaging is likely to deter non-target species, although in some cases non-target species did remove up to 13% of packaged baits.

1. Introduction

In parts of the UK and the Republic of Ireland, the European badger *Meles meles* is a wildlife reservoir for *Mycobacterium bovis* the causative agent of bovine tuberculosis (TB) in cattle (Krebs, 1997; Griffin et al., 2005; Palmer et al., 2012). The parenteral vaccination of badgers with Bacillus Calmette-Guerin (BCG) is one current approach for managing the disease in badger populations, with the aim of reducing infection risk to cattle (Chambers et al., 2014). Badgers can be captured and BCG administered via intra-muscular injection, which has been demonstrated to reduce the severity and progression of disease (Chambers et al., 2011; Carter et al., 2012). An alternative and potentially cheaper approach for widespread vaccine delivery is through oral vaccination, with BCG contained within edible baits (Delahay et al., 2003; Robinson et al., 2012). Oral delivery of BCG to wild possums has been shown to produce a protective response, with reduced bacterial counts compared to control animals (Tompkins et al., 2013). Oral vaccination has also been used successfully to control sylvatic rabies and classical swine fever in wild boar (Rossi et al., 2015).

The development of an oral vaccine requires the design and refinement of several components, including the immunogen itself, the bait/delivery system and the methodology for vaccine deploy-

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ment (Cross et al., 2007). Oral administration to badgers of BCG in solution has been shown to have a protective effect against experimental challenge with *M. bovis* (Murphy et al., 2014), and is currently the only candidate for the oral vaccination of wild badgers (Chambers et al., 2014). As BCG, an attenuated strain of *M. bovis* needs to be alive to be effective as a vaccine, any bait used as a delivery system will need to retain BCG viability up to the point of ingestion.

Badgers live in groups that occupy burrow systems known as setts. A badger oral vaccine will therefore most likely be deployed at badger setts (Delahay et al., 2003; Chambers et al., 2014). Previous research by Robertson et al. (2015) used camera footage to investigate which potential non-target species were present at badger setts and whether they would investigate two simple bait types which are attractive to badgers. This earlier work demonstrated that badger setts are occupied and visited by a wide range of other animal species, particularly rodents which are relatively common and likely to consume deployed baits (Robertson et al., 2015). An oral badger vaccine will ideally consist of a bait and a deployment strategy which together are attractive to badgers, but are ideally less so for non-target species.

To support the development and ultimate licensing of and oral badger vaccine we investigated the behaviour of badgers and non-target wildlife species towards three candidate bait designs for the oral delivery of BCG to badgers. The three candidates varied in their construction and presentation (packaging). Although packaging is unlikely to improve bait attractiveness, it prolongs the environmental stability and integrity of the bait (Gowtage et al., personal communication). Packaging also provides a surface for a written label which is a regulatory requirement for licensing a vaccine. We investigated whether packaging and bait design influenced attractiveness to badgers and non-target species (primarily rodents). By measuring variation in bait removal rates amongst badger setts (social groups) and over time we also obtained information to inform the development of an optimal deployment strategy to maximise bait uptake and vaccine coverage.

2. Materials and methods

2.1. Study area

The field study was carried out over ten consecutive nights from the 6th to the 16th August 2013 in Gloucestershire, southwest England. Sixteen main setts (conspicuous burrow systems occupied by badger social groups) were selected for the study, based on the presence of field signs during preliminary surveys, indicating there were badgers present. The sixteen setts were spread across an area of approximately 170 km^2 (centred around 51°54′N, 2°10′E) where no previous bait, feeding or trapping (for culling or vaccination) studies had been conducted. The habitat within this area consisted predominantly of patches of woodland interspersed with agricultural farmland and is typical of the broad habitat types across much of the area in south-west England in which TB is endemic in cattle.

2.2. Bait options

The study aimed to investigate preference towards three designs for bait presentation; hereafter referred to as 'cup', 'bag' and 'polymer'. Bait designs were based on previous studies using captive (Gowtage et al., personal communication) and wild badgers (Palphramand et al. personal communication) that were aimed at identifying palatable bait formulations and designs. In all cases, the bait consisted of two main components: 1) a peanut-based paste (Connovation Ltd. and Pest Tech Ltd, New Zealand) which is attractive to badgers; and 2) hardened peanut oil (HPO). The 'cup' and 'bag' bait options were of identical design, consisting of a hollow tube of paste bait with a central cylinder of HPO, the total weight of which was approximately 15 g (Fig. 1a). In the 'bag' bait system the bait was contained within a labelled lightweight greaseproof paper bag (140 × 70 mm, Fig. 1a) with two perforations on each side of the bag to release bait odour. Cup baits were contained in an open cardboard cup with a printed paper label attached using cyanoacrylate glue (Fig. 1b). The 'polymer' baits (18 g) comprised the same paste bait mixed with a polymer which hardened the paste into a solid tube which was sealed at one end and filled with HPO. This bait had no packaging but had a paper label wrapped around the outside (Fig. 1c). The ends of the label were attached using cyanoacrylate glue. In addition to the three candidate vaccine bait options, we also deployed a positive control bait consisting of peanuts and syrup (8:1 ratio, in ~15 g portions) that is routinely used in badger bait marking and trapping exercises and is known to be attractive to badgers (Delahay et al., 2000).

2.3. Bait deployment

At each main badger sett, 24 ceramic paving tiles (20 cm x 20 cm, ~2.5 kg) were deployed adjacent to sett entrances and along runs (worn badger paths). A single bait was positioned beneath each tile, thus creating a series of marked bait points and providing some deterrence to smaller non-target species that were unable to move them. A similar approach has been used in other badger bait studies (Kelly et al., 2011). The removal of baits from beneath tiles provided measures of bait selection or preference. This method of delivery was not intended to mimic how a licensed badger vaccine may be deployed, but provided an appropriate experimental design for measuring the relative attractiveness of the three candidate bait designs to wild badgers and non-target species.

Tiles were spaced >5 m apart within 200 m of the sett, and were each marked 1–24 using a waterproof permanent marker pen. All tiles were deployed three to four days before the study commenced to allow resident badgers to become accustomed to their presence. Baits were deployed for ten days, with six of each bait type being placed under randomly allocated numbered tiles on day one of the study. The bait treatment at each tile was rotated daily so that each of the four bait types was placed under each tile two or three times, resulting in 60 of each bait type being deployed at each sett over the ten-day study period. Each bait was placed in a small depression in the ground under a tile at a sufficient depth to avoid it being crushed by the tile. Baits were deployed in the afternoon (to minimise interference from diurnal non-target species) and fresh baits were deployed daily, with any uneaten baits from the previous night being removed. As baits were deployed and removed, whether bait was taken by badgers (tile moved and bait taken) or small mammals (tile not moved, but bait taken or partially consumed) was recorded.

2.4. Video surveillance

In order to provide a relative measure of badger activity, two Bushnell trail cameras (model 119435, Bushnell Trophy camera, UK) were placed at each of the 16 setts, fastened to trees overlooking active areas (either sett entrances or runs). These cameras were motion-activated and set to take 60 s videos whenever activated. Cameras were checked daily.

At all setts, an additional camera was placed overlooking an area with signs of fresh badger activity adjacent to the sett (e.g. near active runs or sett entrances). Four of the 24 tiles (one of each bait type) were deployed in a square configuration, spaced 1 m apart and in view of the camera. At eleven of the setts the camera used was a Bushnell trail camera set to record 60 s videos. At the remaining five setts a video camera (704 by 576 resolution – or higher) with an