Assessment of the safety of Bacillus Calmette-Guérin vaccine administered orally to badgers (Meles meles)

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Abstract
European badgers (Meles meles) are a wildlife reservoir for Mycobacterium bovis (M. bovis) in parts of England, Wales and Ireland, constituting a potential source of tuberculosis (TB) infection for cattle. Vaccination of badgers against TB is one of the tools available for helping reduce the prevalence of bovine TB in badgers, made possible by the licensing in 2010 of Bacillus Calmette-Guérin (BCG) vaccine for intramuscular administration to badgers (BadgerBCG). However, practical limitations associated with administering an injected vaccine to wild animals make an oral, bait-delivered form of the vaccine highly desirable. Evaluation of the safety of oral BCG to badgers and the environment is a mandatory step on the road to licensing an oral vaccine. This study had the following objectives: (a) to determine whether adverse effects followed the oral administration of BCG vaccine to badgers; (b) to measure the quantity and frequency of BCG excreted in the faeces of vaccinated badgers; and (c) to assess whether there was evidence of the vaccine spreading to unvaccinated, ‘sentinel’ badgers housed with the vaccinated animals despite the occasional excretion of BCG in faeces.

1. Introduction

The European badger (Meles meles) was first identified in England as a wildlife host for Mycobacterium bovis, the causative agent of bovine TB, in 1971 [1]. At the present time, populations of badgers in parts of the UK and the Republic of Ireland can act as wildlife reservoirs for M. bovis [2–4], and as such, confound efforts to eradicate bovine TB from these countries.

Vaccinating badgers is one approach to managing the disease in badger populations and part of the TB eradication strategy for England and Wales; feasible since 2010 through the licensing of BadgerBCG, a preparation of the Danish strain of BCG vaccine for intramuscular delivery to badgers [5]. An alternative approach for widespread vaccine delivery to badgers is through delivery of BCG contained within edible baits [6,7]. BCG is protective to badgers by the oral route, most recently demonstrated in a field trial in the Republic of Ireland [8], but it requires higher doses of BCG to be administered compared with the injected vaccine [9,10].
Given the route of vaccination and the fact that relatively high numbers of viable bacteria need to be delivered, administration of BCG orally may lead to adverse effects in the badger and/or a significant quantity of viable vaccine being excreted into the environment, potentially exposing other non-target species to BCG. In particular, the hazard that the vaccine might make its way into cattle thereby compromising the ability to diagnose TB is a concern that needs to be addressed in order for the risk to be properly assessed.

2. Materials and methods

2.1. Ethics statement

All animal procedures were covered by a licence issued by the UK Home Office under the Animal [Scientific Procedures] Act 1986, and approved by the Animal Welfare and Ethical Review Board at the Animal and Plant Health Agency (APHA). This manuscript was prepared to comply with the ARRIVE Guidelines for reporting animal research [11].

2.2. BCG vaccine

BCG vaccine Danish strain 1331 was supplied by the manufacturer (Statens Serum Institute, Denmark) as a suspension in 1.5% (w/v) sodium 2-aminopentanedioate (monosodium glutamate; MSG). The vaccine was stored at 2–8°C and its concentration determined by plating on modified Middlebrook 7H11 agar [12] (BD, Oxford, UK). The intended BCG overdose (6.4 × 10⁹ cfu) was 100 times the intended single dose (6.4 × 10⁷ cfu), which is currently the lowest dose for which we have positive efficacy data (unpublished). To achieve the artificially high concentration of BCG for the overdose, the required volume of vaccine solution was centrifugation at 4600 × g for 20 min at 20°C and the supernatant discarded, leaving a moist pellet of BCG. The single dose was achieved by adjustment of the vaccine solution to a concentration of 3.2 × 10⁶ cfu/ml.

2.3. Badgers

Sixteen badgers were maintained as a confined but free-ranging colony across four discrete pens in isolation from wild badgers in the Natural Environmental Centre (NEC) at APHA. Four were born in captivity to mothers that had been tested and confirmed free of TB. The others were wild-caught animals and determined as free of TB by interferon-gamma release assay [13] and bacteriological culture of clinical samples taken prior to enrolment in the study.

Artificial wooden setts were provided and the badgers used these to rest and sleep in. Use of these setts permitted ready access to the badgers during daylight hours. Throughout the study the badgers were fed dog food and peanuts daily, occasionally chicken eggs, and they were free to forage in the NEC. Mains water was available ad libitum.

For vaccination and/or clinical examination, the badgers were anaesthetised directly in the wooden setts by intramuscular injection of approximately 10 mg/kg of ketamine (Vetalan®, Pfizer Animal Health, New York, NY, USA), 100 μg/kg of medetomidine (Domitor®, Pfizer Animal Health, UK) [14] and 100 μg/kg of butorphanol (Torbugesic®, Zoetis UK Ltd, Tadworth, Surrey, UK), using a pole-syringe (Field Development and Supply, LLC, USA). The badgers were individually identified by unique dorsal fur-clip patterns [15] and by using electronic microchips (IPPT-300 transponders, PLEXX b.v., Elst, Netherlands) placed under the skin dorsally between the shoulder blades. The microchips also allowed for the remote measurement of body temperature, negating the need for any chemical or physical restraint. Microchips were activated and data read by a hand-held reader, DAS-5002 (PLEXX b.v.).

2.4. Allocation to treatment

The study comprised of four groups (Table 1). One badger (#044) had a discharge from its vagina prior to enrolment, so the social group this badger was in was assigned as the control group to minimise the impact on results should antibiotic treatment be required. The remaining social groups were assigned to vaccination. A sentinel animal from within each group allocated to vaccination was chosen to be a sentinel (not vaccinated) by the selection of a different coloured bead taken at random from an opaque container containing three other beads of identical colour.

In recognition that the control group was not assigned by true randomisation we first tested whether this group differed significantly from the other groups for the parameters we used to evaluate vaccine safety: weight, subcutaneous and rectal body temperatures, using ordinary one-way ANOVA. There was no significant difference between the mean weights (p = 0.33, Fig. 1) or the rectal temperatures (p = 0.09, Fig. 3) of the four groups 14 days prior to vaccination, nor between the mean subcutaneous temperatures (p = 0.55, Fig. 2) immediately prior to oral vaccination. For this reason we conclude that the control badgers can be considered to be sampled from the same population as all the other badgers in the study.

Table 1

<table>
<thead>
<tr>
<th>Badger ID</th>
<th>Gender</th>
<th>Group</th>
<th>Treatment</th>
<th>Overdose received (cfu)</th>
<th>Single dose received (cfu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>058</td>
<td>Female</td>
<td>1</td>
<td>Vaccinate</td>
<td>7.90 × 10⁷</td>
<td>7.24 × 10⁷</td>
</tr>
<tr>
<td>366</td>
<td>Female</td>
<td>1</td>
<td>Vaccinate</td>
<td>8.03 × 10⁷</td>
<td>7.24 × 10⁷</td>
</tr>
<tr>
<td>860</td>
<td>Male</td>
<td>1</td>
<td>Sentinel</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>867</td>
<td>Female</td>
<td>1</td>
<td>Vaccinate</td>
<td>7.96 × 10⁷</td>
<td>6.06 × 10⁷</td>
</tr>
<tr>
<td>311</td>
<td>Female</td>
<td>2</td>
<td>Vaccinate</td>
<td>8.04 × 10⁷</td>
<td>6.80 × 10⁷</td>
</tr>
<tr>
<td>525</td>
<td>Female</td>
<td>2</td>
<td>Vaccinate</td>
<td>7.99 × 10⁷</td>
<td>7.24 × 10⁷</td>
</tr>
<tr>
<td>574</td>
<td>Male</td>
<td>2</td>
<td>Sentinel</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>804</td>
<td>Male</td>
<td>2</td>
<td>Vaccinate</td>
<td>8.03 × 10⁷</td>
<td>7.24 × 10⁷</td>
</tr>
<tr>
<td>836 (008)</td>
<td>Male</td>
<td>3</td>
<td>Vaccinate</td>
<td>8.10 × 10⁷</td>
<td>7.24 × 10⁷</td>
</tr>
<tr>
<td>078 (011)</td>
<td>Female</td>
<td>3</td>
<td>Sentinel</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>109</td>
<td>Female</td>
<td>3</td>
<td>Vaccinate</td>
<td>8.10 × 10⁷</td>
<td>7.24 × 10⁷</td>
</tr>
<tr>
<td>793</td>
<td>Female</td>
<td>3</td>
<td>Vaccinate</td>
<td>7.99 × 10⁷</td>
<td>7.24 × 10⁷</td>
</tr>
<tr>
<td>039</td>
<td>Female</td>
<td>4</td>
<td>Control</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>044</td>
<td>Female</td>
<td>4</td>
<td>Control</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>818</td>
<td>Male</td>
<td>4</td>
<td>Control</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>858</td>
<td>Male</td>
<td>4</td>
<td>Control</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*In these cases the original chip failed and was replaced with the ones shown in parentheses.*
the study and hence our conclusions regarding the effects of vaccination are valid.

2.5. Observations

Badger behaviour was monitored by standalone Closed Circuit Television (CCTV) linked to a Pelco DX8100 Series hybrid video recording system (Pelco Corporate, Clovis, CA). Husbandry staff were blinded to the treatment groupings to eliminate this as a potential source of bias regarding behavioural results. The recorded footage was reviewed by experienced staff and the behaviour (normal/abnormal) was recorded. The observations included feeding, interaction with pen-mates and exploration of the pen. In addition to the nocturnal behaviour observations, the badgers were checked at least once daily as part of the normal husbandry routine.

2.6. Vaccination

Three test animals from Groups 1–3 were administered the overdose of BCG orally on Day 0. Each badger was anaesthetised then using a sterile, rounded flexible spatula, BCG was applied to the inside of both cheeks between the outside of the lower molars and the inner buccal surface. A final aliquot was applied at two points underneath and either side of the tongue. The residue of the BCG remaining in the centrifuge tube was re-suspended in 5 ml of sterile PBS and delivered directly into the stomach of the badger via a sterile stomach tube. Whilst still inserted into the stomach the tube was then washed through with a further 5 ml of sterile PBS to remove any residual BCG contained in the tube.

Fourteen days after administration of the overdose the test animals in Groups 1–3 received a single oral BCG dose administered in 50 μl volumes to each of the four locations in the mouth only (200 μl total of the vaccine suspension).

Upon completion of the vaccination procedures, each badger was held in a head-up position to prevent reflux from the oesophagus until there were no visible signs of the vaccine in the mouth and to allow the badger to swallow. This took approx. 30–120 s. The badger was then placed in a holding cage with the head slightly raised to recover from the anaesthetic. The supernatant remaining from the preparation of each overdose was titrated to determine the losses associated with the centrifugation process. Once dosing was complete the residue in each dose container was returned to the laboratory in a portable fridge and titrated to determine the quantity of BCG remaining. From this the dose of vaccine received by each animal was calculated.

2.7. Clinical examinations

At enrolment and at the end of the in-life phase of the study all badgers were subjected to a clinical examination under anaesthesia by a Veterinary Surgeon. In addition, the badgers selected for vaccination were also examined whilst they were under anaesthetic for administration of the BCG doses.
The body temperature of all the badgers in the study was measured using calibrated rectal thermometers and the subcutaneous chip at enrolment, whilst each animal was under anaesthesia and just prior to euthanasia. For the vaccinated badgers only, body temperature was also measured via rectal thermometer and the subcutaneous chip whilst each was under anaesthesia for administration of the BCG overdose and single dose. The body temperature of each badger was also measured daily from day -1 to 46 via the subcutaneous chip only. The body temperature of the vaccinated badgers only was additionally recorded via the subcutaneous chip at 1, 2, 4, 6 and 8 h after each BCG vaccination while the badgers were held individually in cages. The strength of correlation between the temperatures recorded by rectal thermometer versus the subcutaneous chip was tested using Pearson correlation. All statistics performed for this study used GraphPad Prism version 6.04 for Windows (GraphPad Software, La Jolla, California USA, www.graphpad.com).

2.8. Sample collection

On the nights of study days 0, 1, 7, 14 and 21 the vaccinated badgers in Groups 1–3 were housed in individual cages to facilitate the collection of faeces the following morning. Additional, pooled faeces samples were collected from the communal pens of all groups on study days –1 to 48.

Upon completion of collection each day, the faeces samples were immediately transferred to the laboratory. All samples were then split approximately in half and one aliquot of each sample was weighed. The weighed aliquot was subjected to culture for BCG and the remaining aliquot of each sample was immediately stored frozen as a back-up for use in the event of contamination of the first culture.

At the end of the in-life phase of the study (day 119), all of the badgers were euthanased at the NEC over a three day period. Whilst under terminal anaesthesia, just prior to euthanasia, a tracheal aspirate and laryngeal swab sample were taken from each animal. After collection of the swab and aspirate samples, each badger was euthanased using 5 ml of Somulose® (Dechra Veterinary Products, Hadnall, UK) delivered intravenously. Each carcass was transferred to a post-mortem facility for examination and sampling.

The following tissues from all animals were examined macroscopically and samples collected for BCG culture and histology: lymph nodes (tonsils, retropharyngeal, hepatic, mesenteric, anterior and posterior mediastinal, left and right bronchial); mucosa of the gastric fundus, duodenum and ileum walls, colon and rectal-anal junction; liver; spleen; and lung. In addition, a laryngeal swab was taken, as well as samples of faeces from the rectum and urine from the bladder. The following tissues were examined macroscopically only: heart; adrenals; thymus; reproductive organs; thyroid/parathyroid; eyes; ears; pancreas; kidney; muscles, bones, and nerves (brachial and sciatic plexus).

All tissue samples collected at post-mortem for BCG culture were weighed immediately after collection. The samples were then transferred to the laboratory and stored frozen. All tissues for histology were retained securely in buffered formalin in the post-mortem facility for at least 21 days before processing for H&E and ZN staining, according to APHA Standard Operating Procedures. The stained sections were examined microscopically; the H&E sections for general tissue abnormalities and the ZN tissues for the presence of Acid Fast Bacteria (AFB).

Samples of gastric content were taken and weighed upon receipt in the laboratory prior to being stored frozen. Urine and tracheal swab samples collected at post-mortem were transferred to the laboratory for immediate BCG culture, with the exception of samples from two animals that were stored refrigerated overnight and submitted for BCG culture the following morning.

2.9. BCG culture

Saline solution (0.85% w/v) was added to each sample of faeces to form a slurry during retention overnight at +4 °C. Approximately 15 ml of each sample was transferred to a sterile tube and decontaminated using 10% (w/v) ethanedioic acid (oxalic acid). The oxalic acid solution was added up to the 30 ml mark on the tube. After 10 min of contact time, the sample was centrifuged (10 min 1100g) and the supernatant discarded, retaining the pellet. If no discernible pellet had formed, approx. 1 cm depth of supernatant was left in the bottom of the tube. Each tube was then filled to the 30 ml mark with 0.85% saline and secured before vortex mixing until the pellet was re-suspended. The centrifugation step was repeated and the tube filled finally with 0.85% saline to the 10 ml mark. The pellet was re-suspended by vortex mixing. These samples constituted the inocula for sowing onto plates.

All tissues were aseptically transferred to a sterile IKA homogeniser tube (IKA® England Ltd, Oxford, UK). Ten ml of 0.85% (w/v) saline was then added to each tube and the sample homogenised using an ULTRA-TURRAX® Tube Drive (IKA® England Ltd).

Each prepared sample, was inoculated onto a total of six modified 7H11 agar plates (200 µl onto each plate, three plates from two different batches).

All swabs and tracheal aspirates were submitted for culture in 3.5 ml of Middlebrook 7H9 medium (BD, Oxford, UK). Upon receipt in the culture laboratory the swab samples were vortex mixed and 200 µl was inoculated onto a total of six modified 7H11 agar plates (three plates from two different batches). The tracheal aspirates were immediately inoculated onto modified 7H11 agar plates. All plates were incubated for 12 weeks at approximately 37 °C and were checked for any signs of contamination by non-mycobacterial species after six weeks. At the end of the incubation period the plates were examined and the number of BCG colonies present recorded.

3. Results and discussion

3.1. Observations and clinical examinations

All badgers exhibited normal behaviour in respect of their eating, exploring their pen and interactions with pen-mates. All badgers put on weight during the study (Fig. 1) and there was no evidence of any effect of vaccination on their weight.

Subcutaneously implanted chips were used to measure body temperature without needing to disturb the badgers (Fig. 2, Table 2). An increase in body temperature was measured for all of the vaccinated badgers after administration of BCG, reaching a maximum mean temperature four hours post-vaccination. The maximum body temperature of an individual animal after administration of the BCG overdose was 41.8 °C, two hours post-

<table>
<thead>
<tr>
<th>Day</th>
<th>Vaccinated (n = 9)</th>
<th>Controls (n = 4)</th>
<th>Sentinels (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37.4 (36.1–38.8)</td>
<td>36.9 (35.9–37.6)</td>
<td>38.0 (37.1–38.5)</td>
</tr>
<tr>
<td>1</td>
<td>35.6 (33.5–38.1)</td>
<td>36.3 (35.7–36.7)</td>
<td>34.1 (33.5–34.7)</td>
</tr>
<tr>
<td>14</td>
<td>36.6 (34.9–37.7)</td>
<td>36.7 (36.0–37.7)</td>
<td>37.0 (36.2–37.7)</td>
</tr>
<tr>
<td>15</td>
<td>36.2 (34.6–39.5)</td>
<td>36.2 (35.0–36.9)</td>
<td>35.6 (34.5–36.2)</td>
</tr>
</tbody>
</table>
vaccination. The maximum body temperature of an individual animal after administration of the BCG single dose was 40.2 °C, four hours later. The maximum increases in body temperature after administration of the BCG overdose and single dose were 3.4 °C and 3.2 °C, respectively. Twenty-four hours after administration of either the overdose or single dose one badger had a temperature greater than 38 °C (38.1 °C and 39.5 °C, in each case), but this was a different badger on each occasion.

Body temperature measurements made using rectal thermometers (Fig. 3) were higher than those recorded using subcutaneous chip. Across 50 paired measurements the average difference in temperature was +2.8 °C (0.8–6.5 °C) with good correlation between the two \( (r^2 = 0.69, p < 0.0001, \text{Pearson correlation}) \). Over the course of the study the rectal temperature decreased from a mean of 38.7 °C on enrolment to a mean of 37.4 °C at the end of the study, whereas the subcutaneous chip temperature decreased from a mean of 36.0 °C to 33.6 °C. The reduction in body temperature would appear to be part of the normal physiology of badgers, wherein a reduction in body temperature occurs during the winter months and is more influenced by the length of daylight than changes in ambient temperature [16,17]. The greater reduction in subcutaneous chip temperature compared to rectal temperature from the beginning (summer) to the end (winter) of the study suggests that as well as recording the actual seasonal reduction in body temperature, readings from the subcutaneous chip were additionally influenced by ambient temperature, such that the rectal temperature is likely to be a more reliable indicator of the true body temperature.

The average maximal body temperatures seen in this study were very similar to those reported by us for parenterally administered BCG [18], with the exception that the temperatures for half of the animals in this study recorded within two hours of receiving the highest dose of BCG orally were greater than we have seen before. This might reflect a dose-related febrile response. The mean body temperature at this time point was also comparable to that of the controls and sentinels for the majority of the animals. As neither the control nor sentinel animals were subjected to anaesthesia at the times the other badgers were vaccinated we cannot definitively attribute the transient febrile response to vaccination and not anaesthesia. However, the anaesthetic cocktail we used has not previously been reported to affect the rectal temperature of badgers during the process of anaesthesia [19]. We consider it most likely that the transient rise in body temperature was associated with BCG vaccination, consistent with the delivery of an immunogen [20]. More importantly, the mean body temperature of the orally vaccinated badgers returned to, or fell below, the pre-vaccination temperature by 24 h after administration of both the BCG overdose and the single dose.

3.2. Bacteriology

The concentration of BCG on receipt was \( 4.35 \times 10^6 \) (3.40–5.30 \( \times 10^6 \) cfu/ml). Table 1 summarises the treatment allocations, and where appropriate, the dose of BCG received for each badger in the study. The target BCG overdose was \( \geq 6.4 \times 10^8 \) cfu per badger and all badgers received an overdose greater than the target. The target BCG single dose was \( \geq 6.4 \times 10^7 \) cfu per badger. With the exception of one animal (#867) all badgers received a single dose greater than the target. The dose received by badger #867 was still of the same order of magnitude as the target dose (6.06 \( \times 10^7 \) cfu).

During the in-life phase of the study, forty-five pooled faeces samples were collected from each of the pens housing vaccinated badgers. A total of 14 individual faeces samples were taken from the sentinel animals on the occasions that vaccines were in cages. A total of 39 individual faeces samples were collected from the vaccinated badgers whilst they were in cages. In total, BCG was cultured from only three samples: vaccinate #793 on the morning of day 2 (996 cfu/g); vaccinate #867 on the morning of day 2 (372 cfu/g); vaccinate #311 on the morning of day 15 (80 cfu/g), one day after administration of the single dose.

BCG was not cultured from any of the laryngeal swab or tracheal aspirate samples collected under terminal anaesthesia, nor from tissues, tracheal swab, faeces, urine and gastric content collected post mortem. We reported the same when evaluating BCG administered parenterally to badgers [18]. Our ability to recover BCG is least successful from urine (limit of detection of 40 cfu/ml), compared with a limit of detection of 24 cfu/ml from faeces and 4 cfu/ml from laryngeal swab or tracheal aspirate [18].

Our results compare very closely to a previous study in badgers in which \( 10^8 \) cfu BCG Pasteur was administered orally in a lipid matrix [21]. In that study BCG was similarly recovered rarely from faeces taken from the pens of vaccinated badgers up to 17 days post-vaccination. A maximum of 20 cfu/g of faeces was recovered three days after vaccination.

The excretion of BCG from other species when administered orally has been assessed by others and is summarised effectively by Beltrán-Beck et al. [22]. These studies are difficult to compare with each other directly as they differ in the dose and strain of BCG used and the means of its administration (e.g. lipid formulation, bait, etc.), as well as the sampling regime and means of detection employed. In general, a picture emerges of no to little excretion of BCG. When excretion does occur it is several orders of magnitude lower than the administered dose and infrequent [22,23]. One exception might be white-tailed deer (Odocoileus virginianus) in which there is evidence of transmission of BCG from vaccinated deer to other in-contact deer, but not cattle, on the basis of immune responses [24], although BCG could not be recovered by culture. This might be a consequence of the relatively high dose of BCG administered (10⁶ cfu in a lipid matrix) and/or the propensity of BCG to persist in this species and even to disseminate and cause microscopic lesions [25].

3.3. Post-mortem examination

All tissues examined post mortem were considered to be grossly normal. The absence of BCG in the tissues determined by culture was consistent with the histological examination of the same tissues; no AFB were observed. For each animal, the ileum wall, rectal-anaI junction, posterior mediastinal lymph node and lung samples were considered normal. Active follicles were found in different lymphoid tissues from a number of badgers regardless of whether they were vaccinated or controls, so these changes could not be attributed specifically to vaccination. This indicates that activated lymphoid follicles are normal for badgers, as they are for other wildlife species (Salguero, personal communication).

4. Conclusion

No apparent adverse effects arose from the administration of \( \geq 6.4 \times 10^8 \) cfu BCG orally to badgers. A short-lived febrile response was recorded in the majority of vaccinated badgers but did not appear to have any detrimental effect. We did not isolate BCG from the tissues of badgers at post-mortem 120–121 days after the badgers had received the overdose of BCG (106–107 days after the single dose). The only occasions when BCG was recovered from excreta was within 48 h of vaccination and then from only 3/9 animals with a maximum yield of 996 cfu/g of faeces. The lack of apparent vaccine spread to sentinel animals housed together with vaccinated badgers suggests that environmental contamination following BCG ingestion by badgers will be very low, even when high doses of BCG are consumed. As it took oral doses of BCG in
excess of 10^8 cfu to sensitize cattle to the caudal fold tuberculin test [26], the risk of creating false sensitivity in cattle due to BCG excreted from badgers would appear extremely low. The greater risk is that non-target species will consume vaccine baits, as non-target species have been observed to consume candidate badger baits [7,27]. The risk of cattle consuming vaccine baits directly can be reduced through vaccine bait deployment protocols that minimise the opportunity for cattle to access the bait; a strategy shown to deter most non-target species with the exception of rodents [27].

Conflict of interest statement

None.

Acknowledgements

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