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APHA report of examination for *Mycobacterium bovis* in badgers found dead within the Welsh Government Intensive Action Area (IAA) (OG0145/TBOG0146)

Report for project OG0145/TBOG0146

(Year 4)

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Animal and Plant Health Agency (APHA) report on examination for bovine tuberculosis in badgers found dead within the Welsh Government Intensive Action Area (IAA) (OG0145/TBOG0146)

1. SUMMARY

28 found dead badgers were received by Animal and Plant Health Agency (APHA) for examination between 1st May 2015 and 30th April 2016. Of these, 25 were suitable for sampling and *Mycobacterium bovis* was isolated from one of these animals. Three badgers had gross lesions suggestive of tuberculosis (TB) but *Mycobacterium bovis* was not isolated from any of these animals. In years two to four of this survey the proportion of badgers culture positive has been lower than in year one.

2. INTRODUCTION

The Intensive Action Area (IAA) is an area of approximately 288km^2, primarily located in north Pembrokeshire and also including small parts of Ceredigion and Carmarthenshire. A five year badger vaccination project, using injectable Bacillus Calmette-Guérin (BCG) vaccine, began in May 2012. The project was suspended in December 2015 due to interruption of vaccine supplies. Additional cattle controls were also implemented in the IAA with the aim of eradicating bovine TB. A badger found dead survey within the IAA started in June 2012. This reports the results of the survey for carcases received between 1st May 2015 and 30th April 2016.

3. MATERIALS AND METHODS

Locating and collecting badgers

Reports of badgers found dead within the IAA were received by the Welsh Government who collected and delivered the carcases to the APHA Veterinary Investigation Centre at Carmarthen. From the 1st January 2016 reports were received and the badgers were then collected and delivered by APHA Field Services. Carcases were double bagged on collection and, when delivered to APHA, were accompanied by a submission form which included a map reference indicating where the badger was found. On receipt, carcases were stored at between 2°C and 8°C until examined. Examinations were carried out as soon as possible and within no more than four days after receipt.

***Post mortem* examination and sampling at APHA**

Carcases were considered unsuitable if:

- viscera herniated externally through wounds
- there was severe myiasis (flystrike)
- the carcase was distended with gas
- the carcase was flattened

The necropsy consisted of an external examination, which included an examination for bite wounds, evidence of vaccination and evidence of illegal interference and an internal examination of lymph nodes and viscera for lesions of possible tuberculosis. A fresh set of sterilised instruments and disposable gloves were used for each carcase. The skin was reflected along the ventral mid line and the abdominal and thoracic cavities were opened. The following lymph nodes and organs were examined for tuberculous lesions:

- Submaxillary
- Retropharyngeal
- External cervical
- Axillary
- Bronchial
- Mediastinal
- Hepatic
- Renal (if visible)
- Mesenteric
- Gastric
- Internal iliac
- External iliac
- Superficial inguinal
- Popliteal
- Lungs
- Pericardial sac
- Liver
- Kidneys

Each lymph node was incised at least once. An external examination of the lungs, pericardial sac, liver and kidneys was carried out. The lungs were examined by making multiple longitudinal incisions approximately one centimetre apart. At least four slices were made in the liver and three slices in the kidney.

The result of examination for guard hair clip marks and coloured spray on the back of the badger was recorded (these are used in the field to temporarily identify badgers which have been vaccinated). Also an examination of the muscles of the anterior thigh was carried out and the presence of any possible vaccine associated lesions recorded.

Tissues for culture for *M. bovis* were placed in a universal container containing 15ml of 1% aqueous cetylpyridinium chloride. From all suitable badgers, a pool of lymph nodes (pool 1) was collected consisting of the retropharyngeal, bronchial, mediastinal

and hepatic lymph nodes (or as many as were detectable). If any gross internal lesions suggestive of tuberculosis were found on examination of four organs and a further ten lymph nodes (see above), or bite wounds were detected, the lesioned tissue and/or excised bite wounds were added to a separate universal container (pool 2). Samples were sent to APHA Starcross on the day of examination, for next day receipt.

Culture, molecular typing and histological examination

On receipt at APHA Starcross, the tissues were washed in sterile 0.85% saline solution, homogenised by standard methods, inoculated onto 6 Modified 7H11 agar slopes and incubated at 37°C. Pool 1, and Pool 2 (if collected), were cultured separately. The slopes were examined weekly from the end of week two for a maximum of 12 weeks. Isolates were harvested when colonial growth was sufficient for genotyping and sent to APHA Weybridge.

Genotyping was performed using spoligotyping (Kamerbeek and others 1997) and VNTR typing (Exact Tandem Repeat loci A to F, Frothingham and Meeker-O'Connell 1998). Spoligotyping confirmed that the isolates were *M. bovis*. Genotypes of *M. bovis* were labelled according to the current APHA convention, using numbers to represent spoligotypes and lower case letters to represent the VNTR pattern within each spoligotype.

Data management

The information from carcase collection forms and necropsy forms, and results of culture for *M. bovis*, spoligotyping and VNTR testing were recorded on a Microsoft Access database at APHA Weybridge.

4. RESULTS

Of the 29 calls received by Welsh Government eight badgers were not collected as seven were deemed unsuitable and one was not found. Of the 15 calls received by APHA eight badgers were not collected as they were either not found, deemed unsuitable or there was no resource to collect. Badgers deemed unsuitable for collection as part of this project were reported to the County Council for collection and disposal. Of the 28 carcases submitted to APHA from the IAA between the 1st of

May 2015 and 30th April 2016, 25 were considered suitable for sampling. One of the 25 badgers sampled was positive for *Mycobacterium bovis*. The results of examination and culture are in Table 1 below:

Table 1: Results of necropsy and culture for *Mycobacterium bovis* of badger carcases received at APHA from the IAA (May 1st 2015 to April 30th 2016).

No. of carcases reported to Welsh Government or APHA from the IAA that were seen	44		
No. of carcases received by APHA	28		
No. of carcases sampled	**Total**	Tissue pool 1 only	Tissue pool 1 and 2
	25	20	5
No. with bite wounds	5		
No. with gross lesions of possible tuberculosis	4		
No. with lesion(s) in the anterior thigh muscle	0		
No. with evidence of illegal interference	1*		
No. with evidence of trap related injury	0		
No. carcases culture positive for *Mycobacterium bovis* from tissue Pool 1	1		

The results of molecular typing of the two *M. bovis* isolates are in Table 2 below:

Table 2: Results of molecular typing of One isolate of *Mycobacterium bovis* from a badger carcase from the IAA (1st May 2015 to 30th April 2016).

Spoligotype	VNTR	Genotype	No. of isolates
9:	7-5-5-5*-2-2.1	**9:an**	**1**

Notes*: One badger submitted had a snare around its neck and the incident was reported to Dyfed-Powys Police.

The locations of badger carcases that were suitable for examination are in Figure 1 below:

Figure 1: Map of locations of found dead badgers from which culture for *Mycobacterium bovis* was carried out.

Please note some badgers were found in the same location and therefore are not visible as separate dots.

5. COMMENTS

1. In year one of this study, seven of the 37 badgers' sampled (95% confidence interval (CI) 8-35%) were positive for a field strain of *M. bovis* (excluding likely BCG). In Year two of this study, two of the 30 badgers sampled (95% CI 1-22%) were positive for a field strain of *M. bovis.* In year three, two of the 31 badgers sampled (95% CI 0.8-21.4%) were positive for a field strain of *M. bovis.* In year four, one of the 25 badgers sampled (95% CI 0.1-20.4%) was positive for a field strain of *M. bovis*.
2. The small number of badgers found dead, mainly killed due to road traffic accidents, and their TB infection status may not be representative of the badger population and TB prevalence within the IAA. However, as the sampling method is consistent between years they can be considered comparable.
3. The methods used were similar to the standard protocol used in several other studies (Crawshaw et al 2008), but with the addition of the hepatic lymph node to the standard pool (pool 1 in this study) and an extended culture time of 12 weeks to improve sensitivity of culture (Crawshaw et al 2008).
4. The marking of badgers by clipping guard hairs and spraying with a coloured marker spray will identify vaccinated badgers for a limited time only. The absence of visible spray and clip marks and detectable injection sites does not necessarily mean badgers were not vaccinated. None, some or all of the badgers sampled could have been vaccinated at some point during the four years of this project.
5. The isolate of field strain of *M. bovis* in Year four of this study is genotype 9:an which is very rare and most likely a single VNTR mutant of genotype 9:b. *M. bovis* genotype 9:an has been previously detected in cattle within the IAA (AHVLA 2013) and badgers within the IAA in Year three of this study.
6. Seven of 37 badgers were positive for M. bovis in year one, but only five of 96 badgers were positive for M. bovis in years two to four. The contrast between year one and years two to four is marginally significant by a two-tailed Fisher's exact test (P=0.037). However, the proportion of culture positive badgers may change between years for reasons independent of external interventions.

7. In a larger study examining found dead badgers from the whole of Wales between 26 October 2005 and 31 May 2006, 12% of carcases were culture positive; but this incorporated submissions from low cattle TB incidence areas. The nine of 63 badgers found culture positive in Pembrokeshire in the 2005-6 study is consistent with seven of 37 badgers being culture positive in year one of the IAA study (Fisher's exact test P=0.580), whereas five of 96 (95% CI 1.7-11.7%) badgers culture positive in years two to four is significantly less than 12%. Although the IAA study is limited to a relatively small area compared with the 2005-6 study, comparison with the earlier study suggests that the proportion of badgers positive in year one of the IAA study was not unusually high.

6. REFERENCES

AHVLA (2013) Bovine tuberculosis: infection status in cattle in GB p 72. Available at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/413806/tb-pub-surveport-gb13.pdf

CRAWSHAW, T. R., GRIFFITHS, I. B. & CLIFTON-HADLEY, R. S. (2008) Comparison of a standard and a detailed postmortem protocol for detecting *Mycobacterium bovis* in badgers. Vet Rec 163, 473-477

FROTHINGHAM, R. & MEEKER-O'CONNELL, W. A. (1998) Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. Microbiology 144 (Pt 5), 1189-1196

GOODCHILD, A. V., WATKINS, G. H., SAYERS, A. R., JONES, J. R. & CLIFTON-HADLEY, R. S. (2012) Geographical association between the genotype of bovine tuberculosis in found dead badgers and in cattle herds. Vet Rec 170, 259

KAMERBEEK, J., SCHOULS, L., KOLK, A., VAN AGTERVELD, M., VAN SOOLINGEN, D., KUIJPER, S., BUNSCHOTEN, A., MOLHUIZEN, H., SHAW, R., GOYAL, M. & VAN EMBDEN, J. (1997) Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 35, 907-914

7. ACKNOWLEDGEMENTS

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