Environment, Planning and Countryside Committee

EPC(2) 12-06(p2)

Date:	28 September 2006
Venue:	Committee room 2, Senedd
Title:	Badger Found Dead Survey Report

Purpose

1. To provide EPC Committee with an update on available results from the Badger Found Dead Survey.

Recommendations

2. The Committee is asked to note the available results from the Badger Found Dead Survey as per the report from the Veterinary Laboratories Agency (VLA) at Annex 1.

Background

3. A principal recommendation of the EPC Committee Inquiry into TB was the establishment of a Wales TB Action Group to discuss and provide advice to me on the implementation of the Inquiries recommendations and to address specific issues associated with TB. The Group, which is chaired by the Deputy Minister, includes representatives from FUW, NFU Cymru, Country, Land and Business Association, Young Farmers Clubs Wales, RSPCA Cymru, Wales Environment Link, CCW, British Cattle Veterinary Association and State Veterinary Service in Wales.

4. In response to their recommendations I announced on 15 December 2005 a number of measures to address bTB in Wales, including activity associated with wildlife i.e. an all Wales survey of badgers found dead to establish whether they are carrying bTB and a regional survey of badger populations.

5. I informed EPC Committee in May that the level of co-operation from the farming community, Local Authorities and general public to the request to notify us of badgers found dead had been extremely successful and that the survey had received more reports in four months than expected in a year. I therefore announced the end of the survey and agreed that available results would be presented to EPC Committee on 28 September 2006. The report by VLA on the results from the survey is attached at Annex 1.

6. As per my announcement at the Royal Welsh Show on 25 July, I have provided the reports from the Badger Found Dead Survey and regional badger population survey to the members of the Wales TB Action Group for their consideration and to help inform their on-going advice to me.

7. I will ensure that the report from the Found Dead Survey is made available to Committee members and the Wales TB Action Group on completion in January 2007.

Committee Action

8. The Committee is asked to note the detailed interim report from VLA at Annex 1.

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Veterinary Laboratories Agency

Survey of *Mycobacterium bovis* infection in badgers found dead in Wales

Interim report for project OG0017 - Bovine tuberculosis: Pathological and microbiological support for the Welsh Assembly Government's Found Dead Survey

Date of report: 8 September 2006

Interim report for project OG0017 – Bovine tuberculosis: Pathological and microbiological support for the Welsh Assembly Government's Found Dead Survey

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1. SUMMARY

Between 26 October 2005 and 31 May 2006, 549 found dead badgers were submitted to Veterinary Laboratories Agency (VLA) Regional Laboratories, 459 of these were considered suitable for examination and 457 cultured for mycobacterial species. As at September 2006, *Mycobacterium bovis* (*M.bovis*) has been isolated from samples collected from 55 badgers (12%) with 1 culture result pending. Histological examinations indicate that a further two badgers were infected with organisms resembling *M. bovis*.

2. INTRODUCTION

Between 26 October 2005 and 31 May 2006, a survey was carried out of *M. bovis* infection in badgers found dead in Wales. The aims of the study were to:

- Estimate the prevalence of *M. bovis* infection in badgers found dead in Wales.
- Describe the geographical distribution of *M. bovis* positive and negative badger carcases.
- Determine the molecular types of *M. bovis* cultured from badger carcases and geographical distribution of these molecular types.
- Investigate the value of the lateral-flow immunoassay for the diagnosis of *M. bovis* infection in badgers found dead.

This report presents all results available up to 8 September 2006. A final report of all results and analyses will follow in January 2007.

3. MATERIALS AND METHODS

3.1 Reporting and collections

Telephone reports of badgers that were found dead were made to the State Veterinary Service (SVS). SVS staff visited the site and collected badger carcases that were suitable for further examination. Carcases were not

collected if it was considered dangerous to do so. A carcase was considered unsuitable if one or more of these 4 criteria applied:

1. A body cavity was not intact.
2. The carcase was distended with gas.
3. The carcase was flattened.
4. The carcase was flyblown.

A carcase report form (see Appendix 1) was completed for each carcase reported, whether or not it was found or collected. Suitable carcases were placed in 2 sealed plastic bags and the outer bag identified with a carcase label having a unique identification number. The carcases were delivered to the laboratory, usually within 24 hours of collection. Most carcases were not refrigerated between collection and delivery.

3.2. Examination of carcases at the laboratory

The carcases were stored in a refrigerator at between 2 ^{o}C and 8^{o}C after delivery. An examination was carried out to confirm that the carcase was suitable using the same exclusion criteria as at collection. The post mortem examination was carried out as soon as possible after delivery and always within 72 hours.

3.2.1 Collection of blood and use of the lateral-flow immunoassay to detect antibodies to *M. bovis*

Where possible, a blood sample was collected for the lateral-flow immunoassay, either by using a syringe and 14-gauge needle from the thorax via the chest wall before the carcass was opened or, if this was not possible, during the necropsy. An assessment of the condition of blood was made on a scale of one to four with one being fresh with little haemolysis, and four being severely autolysed and haemolysed. The lateral-flow immunoassay (Brock TB Stat-Pak, Chembio Diagnostic Systems, Inc) was carried out according to the manufacturers' instructions.

3.2.2 Necropsy and sampling

An external examination for bite wounds and signs of illegal interference was carried out. The weight, sex and carcase length from nose tip to tail base were recorded. An assessment of age was made by examination of the incisor teeth.

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 lesions suspicious of tuberculosis:

- Submaxillary
- Retropharyngeal
- Prescapular
- Axillary
- Bronchial
- Mediastinal
- Hepatic
- Renal (if visible)
- Mesenteric
- 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. From all carcases that were examined, it was attempted to collect a pooled sample of one half of each of the retropharyngeal and bronchial lymph nodes and half of the mediastinal lymph nodes (half of the hepatic lymph node was collected after 15 March 2006 because a recent analysis of VLA data [unpublished] had shown acid fast organisms occurred more frequently in the hepatic than other lymph nodes not included in the standard sample: therefore inclusion of this lymph node in the standard sample could increase sensitivity of *post mortem* detection of tuberculosis in badgers). In some badgers, it was not possible to collect a full range of lymph nodes, as they were not all identifiable.

The lymph nodes were pooled in universal tubes containing 15ml of 1% aqueous cetylpyridinium chloride (CPC). If a lesion was seen that was possibly due to mycobacterial infection (visible lesion), a sample of it was added to the pooled sample and if the lesion was of sufficient size, a portion was preserved in 10% buffered formalin. If bite wounds were identified, half of each bite wound was collected for culture in a separate universal tube containing 15 ml of 1% CPC and the other half was fixed in 10% buffered formalin. The necropsy findings were recorded on form WA1 (see Appendix 2) and the culture and histopathology results on form WA2 (see Appendix 3).

3.2.3 Culture for *Mycobacterium bovis* and histological examination

Samples in CPC were kept at room temperature and sent to the mycobacterial culture laboratory on the day of collection. On arrival at the culture laboratory, usually the next day, samples were removed from CPC, washed in sterile 0.85% saline, homogenised by standard methods and inoculated onto 12 Modified Middlebrook 7H11 agar slopes. Cultures were incubated at 37^{o}C and examined weekly after the second week for up to 12 weeks. Any growth which appeared to be a mycobacterial species was harvested and submitted for confirmation and identification by spoligotyping.

If lesions were seen suggestive of tuberculosis but *M. bovis* was not isolated from the tissue pool, a Ziehl Neelsen stained section of the formalin fixed lesion (if available) was examined for acid-fast organisms resembling *M. bovis*. If *M. bovis* was not isolated from a bite wound, a Ziehl Neelson stained section was prepared of the fixed bite wound sample (if available) from the same badger and examined for acid-fast organisms resembling *M. bovis*.

4. RESULTS (as at 8 September 2006)

The results available on 8 September 2006, summarising all the badgers examined and all found infected are shown in Table 1 and in Table 2 for each county / unitary authority. These culture results are also presented on a map (Figure1). The results of the lateral - flow immunoassays and of VNTR typing, which are still pending, will follow in the final report.

Table 1. Summary of available results for badger carcases submitted to VLA September 2006

1	Number submitted to laboratories	**549**	
2	Number not suitable for examination	**90** [a]	
3	Number examined	**459**	
4	Number cultured for mycobacteria	**457** [b]	
5	Number with lesion(s) suspicious of *M. bovis* infection	**88 (19%)**	
6	Number with bite wounds	**101 (22%)**	
7	Number positive for *M. bovis* by culture	**55 (12%)**	
8	Number of badgers infected with each spoligotype of *M. bovis*	**Spoligotype 9**	**34**
		Spoligotype 17	**11**
		Spoligotype 22	**10**
9	Number negative by culture for *M. bovis*	**401**	
10	Number culture result pending	**1**	
11a	Number with visible lesion, culture negative	**66** [c]	
11b	Number (of 11a) that are histologically positive	**2**	
11c	Number (of 11a) that are histologically negative	**49**	

Notes:

a. The carcases were examined at VLA to assess whether they were suitable for the study (see section 3.2). The reasons for unsuitability were not recorded.

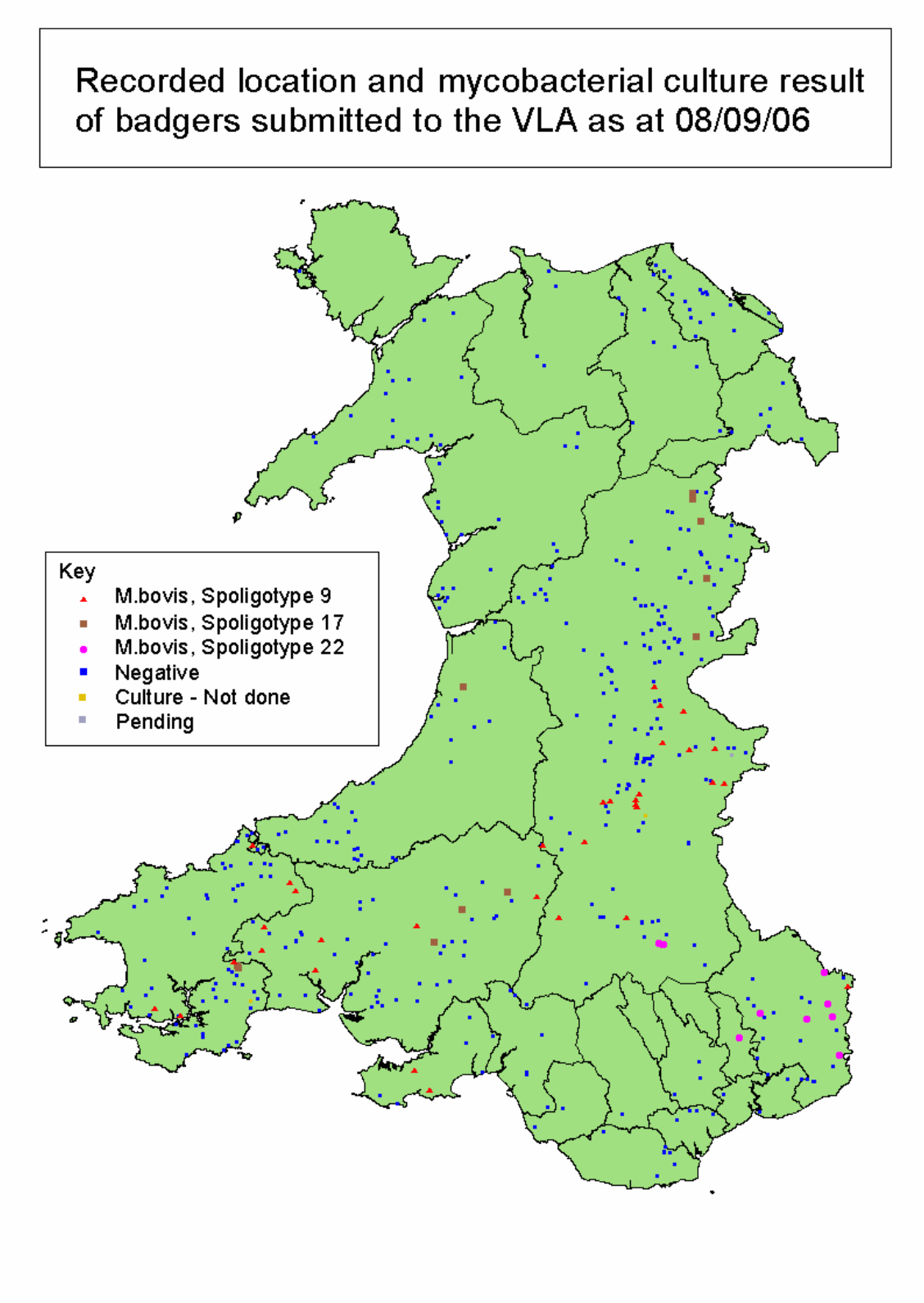
b. Two carcases were initially considered suitable for examination by the criteria previously described, but none of the lymph nodes in the standard sample were identifiable, so no culture was carried out.

c. It was not possible to collect a histological sample of some very small visible lesions and priority was given to sampling for culture. Therefore the sum of 11b and 11c does not equal 11a. Bite wound histology results are not included in this interim report and will be included in the final report.

Table 2 - Results of survey of *M. bovis* infection in badgers as at September 2006

County / Unitary Authority	1. Number examined	2. Number cultured	3. Number positive for *M. bovis* by culture	4. Spoligotypes of *M. bovis* detected		5. Number culture result pending
Anglesey	1	1	0			0
Conwy	5	5	0			0
Denbighshire	9	9	0			0
Flintshire	18	18	0			0
Wrexham	6	6	0			0
Gwynedd	37	37	0			0
Powys	172	171	27	Spoligotype 9	19	1
				Spoligotype 17	5	
				Spoligotype 22	3	
Ceredigion	31	31	1	Spoligotype 17	1	0
Pembrokeshire	63	62	8	Spoligotype 9	6	0
				Spoligotype 17	2	
Carmarthenshire	56	56	9	Spoligotype 9	6	0
				Spoligotype 17	3	
Monmouthshire	25	25	7	Spoligotype 9	1	0
				Spoligotype 22	6	
Swansea	8	8	2	Spoligotype 9	2	0
Neath – Port Talbot	5	5	0			0
Bridgend	2	2	0			0
Vale of Glamorgan	6	6	0			0
Rhondda Cynon Taff	1	1	0			0
Merthyr Tydfil	0	0	0			0
Caerphilly	3	3	0			0
Blaenau Gwent	0	0	0			0
Torfaen	2	2	1	Spoligotype 22	1	0
Cardiff	5	5	0			0
Newport	4	4	0			0
TOTAL	**459**	**457**	**55**			**1**

Figure 1.


Recorded location and mycobacterial culture result of badgers submitted to the VLA as at 08/09/06
Key
M.bovis, Spoligotype 9
M.bovis, Spoligotype 17
M.bovis, Spoligotype 22
Negative
Culture - Not done
Pending

5. DISCUSSION

Evaluation and interpretation by the VLA of the results of survey, as they relate to the four principle objectives, will be included in the final report of the project. This will be produced when all laboratory results are available for analysis and should be submitted in January 2007. Outstanding results include:

- One culture result
- Further histology results
- VNTR types of *M. bovis* isolates
- Results of lateral-flow immunoassay and analysis of degree of correlation with culture results

6. ACKNOWLEDGEMENTS

The VLA acknowledges the contribution of the many organisations and people who collaborated to report and transport dead badgers for examination, and to the Welsh Assembly Government for funding and facilitating this investigation.