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

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

Date of report: 29th January 2007

1. EXECUTIVE SUMMARY

1.1 Between 26 October 2005 and 31 May 2006, 727 badgers found dead in Wales were reported to the State Veterinary Service and 549 found dead badgers were submitted by them to Veterinary Laboratories Agency (VLA) Regional Laboratories. 459 of these were considered suitable for examination and attempted culture for mycobacteria was done on 457.

1.2 For geographical analyses, some Unitary Authorities were aggregated or split into nine geographical units from which between 30 and 88 badgers were submitted. Most badgers were received from Southern Powys (88), Northern Powys (83), Pembrokeshire (62) and Carmarthenshire (56). Fewer were received from North-East Wales, North-West Wales (38 each), Ceredigion, the former Gwent (31 each) and the former Glamorgan (30).

1.3 *Mycobacterium bovis* was cultured from samples collected from 55 badgers. Organisms resembling *M. bovis* were detected histologically in a further six, culture-negative, badgers. Thus 61 badgers were considered positive for bovine tuberculosis, although some of the six badgers that were positive by histology alone could be false-positives.

1.4 Estimates of prevalence of positive badgers varied significantly with geographical unit (Chi-square statistic = 26.59; 8 degree of freedom). Prevalence was highest in Gwent (26%; 95% confidence interval (C. I.) 12–45%), Southern Powys (25%; 95% C.I. 16-35%), Carmarthenshire (16%; 95% C.I. 8-28%) and Pembrokeshire (15%; 95% C.I. 7-26%). It was much lower in the northern part of Wales: Northern Powys (7%; 95% C.I. 3-15%), Ceredigion (3%; 95% C.I. 0.1-17%), North-West Wales (5%; 95% C.I. 0.17-18%) and North-East Wales (3%; 95% C.I. 0.1-14%). Badgers in the latter two geographical units were diagnosed by histology only and were culture-negative. Therefore, they could be false-positives. The difference in prevalence of positive badgers between the geographical units used for analysis was significant. These estimates of prevalence are best interpreted relatively rather than absolutely.

1.5 The 55 *M. bovis* isolates were subjected to two molecular typing tests: spoligotyping and variable number tandem repeats (VNTR) typing. Four molecular

patterns were found. In descending order of frequency, they were spoligotype 9/VNTR 7524*33.1 (20, most from Southern Powys); spoligotype 9/VNTR 7555*32.1 (14, most from Carmarthenshire and Pembrokeshire); spoligotype 17/VNTR 7555*33.1 (11, most from Northern Powys, Carmarthenshire and Pembrokeshire); and spoligotype 22/VNTR 7555*33.1 (10, from Gwent and Southern Powys). The association between molecular type and geographical unit was highly significant ($P < 0.0001$).

1.6   Analysis of molecular typing data of *M. bovis* isolated from <u>cattle</u> in Wales since 2002, showed some similarities between the geographical distribution of molecular types of 989 cattle and the 55 badger isolates. The four molecular types found in 55 found dead badgers were also found in infected cattle. However, 11 molecular types of *M. bovis* were found in cattle but not in these badgers. The size and nature of the sampled badgers does not enable explanation of this finding: it could be that these molecular types exist within the cattle but not the badger population <u>or</u> that had more badgers been sampled, some or all of these types would have been detected.

1.7   The percentage of <u>cattle</u> herds in the nine geographical areas with a confirmed new incident(s) (CNI) of tuberculosis ranged from 0.14% to 6.76% for the duration of the Found Dead Badger Survey and from 0.09% to 4.27% for the period 1/1/2002 to the start of the Survey on 25/10/2005. There was significant correlation between the prevalence of positive badgers and the percentage of CNI in cattle herds for the nine geographical areas for each of these periods. This suggests that there may be common risk factors for *M. bovis* infection of cattle and badgers or cross infection between them, but it does not indicate the direction in which any such transmission occurs.

1.8   The geographical association between *M. bovis* infection of badgers and cattle was examined in two other ways: Firstly, by comparing the number of cattle CNI (from 1/1/2002 to 25/10/2005) within 3 and also 5 km of both positive and negative found dead badgers. There were more CNI of *M. bovis* infection in cattle in areas around positive badgers than in areas around negative badgers, more for 5km (95% confidence interval of the odds ratio was 2.4 to 585) than for 3km (95% confidence interval of the odds ratio was 1.23 to 6.84) radii. Secondly, by comparing the molecular types of three sets of paired results: a badger paired with another badger, a cattle CNI paired with another cattle CNI and a badger paired with a cattle

CNI according to distance apart. At small distances (< 10 km), 70-80% of pairs of samples had the same molecular type, irrespective of whether the samples were both taken from badgers, both from cattle or from one of each species. At moderate distances (40-100 km) badgers were less likely than cattle to have identical molecular types. Both of these analyses suggest that there may be cross infection of *M. bovis* between cattle and badgers but they do not indicate the direction in which any such transmission occurs.

1.9   A lateral flow immunoassay for the detection of antibodies in badger serum to *M. bovis* was evaluated for the first time on blood collected *post mortem* from 379 badgers, of which 42 were subsequently found to be positive and 337 negative for *M. bovis* infection. The test was positive in 13 of the former and four of the latter group. The calculated sensitivity of the test was 31% (95% CI = 17.6-47.0) and specificity was98.8% (95% CI = 96.99 to 99.68) when compared to the standard methods of diagnosis.

1.10   Conclusions

The main findings of this survey are in broad agreement with similar (but not identical) studies of road traffic accident badgers in England and in Northern Ireland. The prevalence of *M. bovis* infection in badgers was highest in areas of high cattle prevalence and lowest in areas of low cattle prevalence.

The results of this survey are consistent with the hypothesis that the badger is an important component in the epidemiology of bovine tuberculosis in areas of high cattle incidence. However, these results do not indicate the major direction of transmission of *M. bovis* between the two species. Nor do they indicate the proportion of infected cattle that acquire *M. bovis* from badgers, from cattle or from other sources.

Tuberculosis in badgers found dead in Wales between 26th Oct 05 and 31st May 06, and groups of Unitary Authorities for which prevalence was calculated. The locations from which the 457 badgers were collected are shown, with laboratory findings. *Culture positive* indicates confirmed infection, and finding *acid fast bacteria* on microscopic examination of visible lesions suggests probable infection.
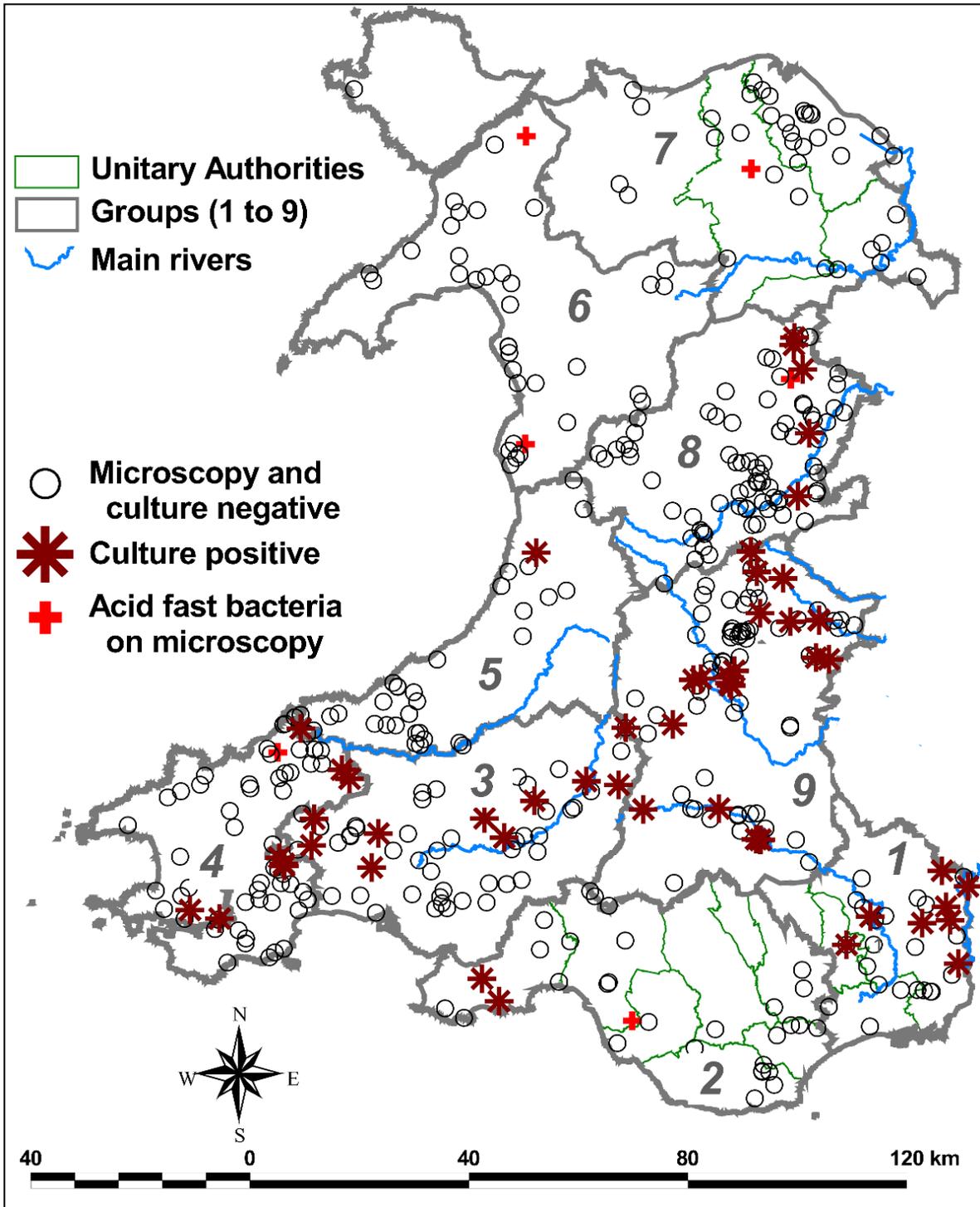
Geographical variation in the prevalence of tuberculosis in badgers in nine groups of Welsh unitary authorities

Group of UAs	Numbers of badgers examined or with molecular types (spoligotype and VNTR type)						Total number infected	
	Number exam-ined	S = 17 7555*3 3.1	S = 22 7524*3 3.1	S = 9 7524*3 3.1	S = 9 7555*3 2.1	Acid-fast organ-isms.	*Number*	Prevalence %, with 95% Conf. interval [1]
1. Gwent	31	0	**7**	1	0	0	*8*	26% (12-45%)
2. Glamorgan	30	0	0	0	**2**	1	*3*	10% (2-27%)
3. Carmarthenshire	56	3	0	0	**6**	0	*9*	16% (8-28%)
4. Pembrokeshire	62	2	0	0	**6**	1	*9*	15% (7-26%)
5. Ceredigion	31	1	0	0	0	0	*1*	3% (0.1-17%)
6. NW Wales	37	0	0	0	0	2	*2*	5% (0.7-18%)
7. NE Wales	39	0	0	0	0	1	*1*	3% (0.1-14%)
8. N'n Powys	83	**5**	0	0	0	1	*6*	7% (3-15%)
9. S'n Powys	88	0	3	**19**	0	0	*22*	25% (16-35%)
Total	457	11	10	20	14	6	*61*	13.3% (10.4-16.8%)

[1] Calculated online using http://statpages.org/confint.html (author JC Pezzullo; accessed 16 January 2007).

The proportion infected varied significantly with group of unitary authorities: Chi-Square Statistic (χ^2) = 26.59 with 8 degrees of freedom (d.f.); right-tail probability (*P*) = 0.0008. The number of badgers with the most prevalent molecular type in each row is shown in **bold**.

The [molecular type] x [group of UAs] interaction was significant: χ^2 = 69.96, 9 d.f., *P* < 0.0001, *after excluding counties with fewer than 8 badgers of known molecular type.*