Mycobacterium bovis surveillance in Eurasian badgers (*Meles meles*) killed by vehicles in Northern Ireland between 1998 and 2011

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Despite extensive long-term eradication programmes, bovine tuberculosis (bTB) remains endemic in much of the British Isles. The cost of the national eradication programme in Northern Ireland was estimated at £23 million in 2010/2011.[1] There is evidence that badgers play a role in the maintenance and spread of *Mycobacterium bovis* to cattle (as reviewed by Allen and others[2]). Northern Ireland is a small country (13,843 km^2) with an agricultural land that is dominated by grass production, which supports 1.6 million cattle among 20,000 farms.[3] The estimated badger population of 34,100 (95 per cent confidence interval (CI) 26,200 to 42,000) is widespread and contained within 7600 social groups (95 per cent CI 6200 to 9000).[4] A road traffic accident (RTA) survey began in 1998 in Northern Ireland with the aim of describing the occurrence of *M bovis* within the badger population.

A wildlife officer and a dedicated collection vehicle were used to collect badger carcases for the survey. All reports of badger carcases found on roads were followed up where possible. To minimise reporting bias, the reporting of carcases was initially limited to the employees of the Department of Agriculture, Environment and Rural Affairs and certain other public sector organisations, but it was later widened to include herd keepers and members of the public. Any carcases found where the cause of death was suspected to be non-accidental were reported to the local police wildlife officer and were excluded from the study. Only carcases deemed suitable for postmortem examination were taken to the nearer of the two veterinary diagnostic laboratories (located in Belfast or Omagh).

Submitted carcases were placed in a class I fume cabinet or on a down ventilated bench, where a detailed postmortem examination was normally carried out within 24 hours of submission (see Fig 1). The sex and approximate age of the badger were recorded, and the carcase was examined for abscesses and wounds. The thoracic and abdominal cavities were opened to expose all organs and lymph nodes, and the skin reflected to expose all head and peripheral lymph nodes. The lymph nodes, liver, kidneys, pericardial sac and pleura were carefully examined for alterations in size and consistency. Multiple incisions were made in the liver, kidneys and lungs, and the cut surfaces were examined. Clotted blood, lymph node pools (prescapular/popliteal; mesenteric; retropharyngeal and mediastinal/bronchial), kidney, urine and faeces were routinely collected for bacteriological culture using aseptic techniques where possible (see Table 1). The spleen was taken as part of the routine sampling at the very

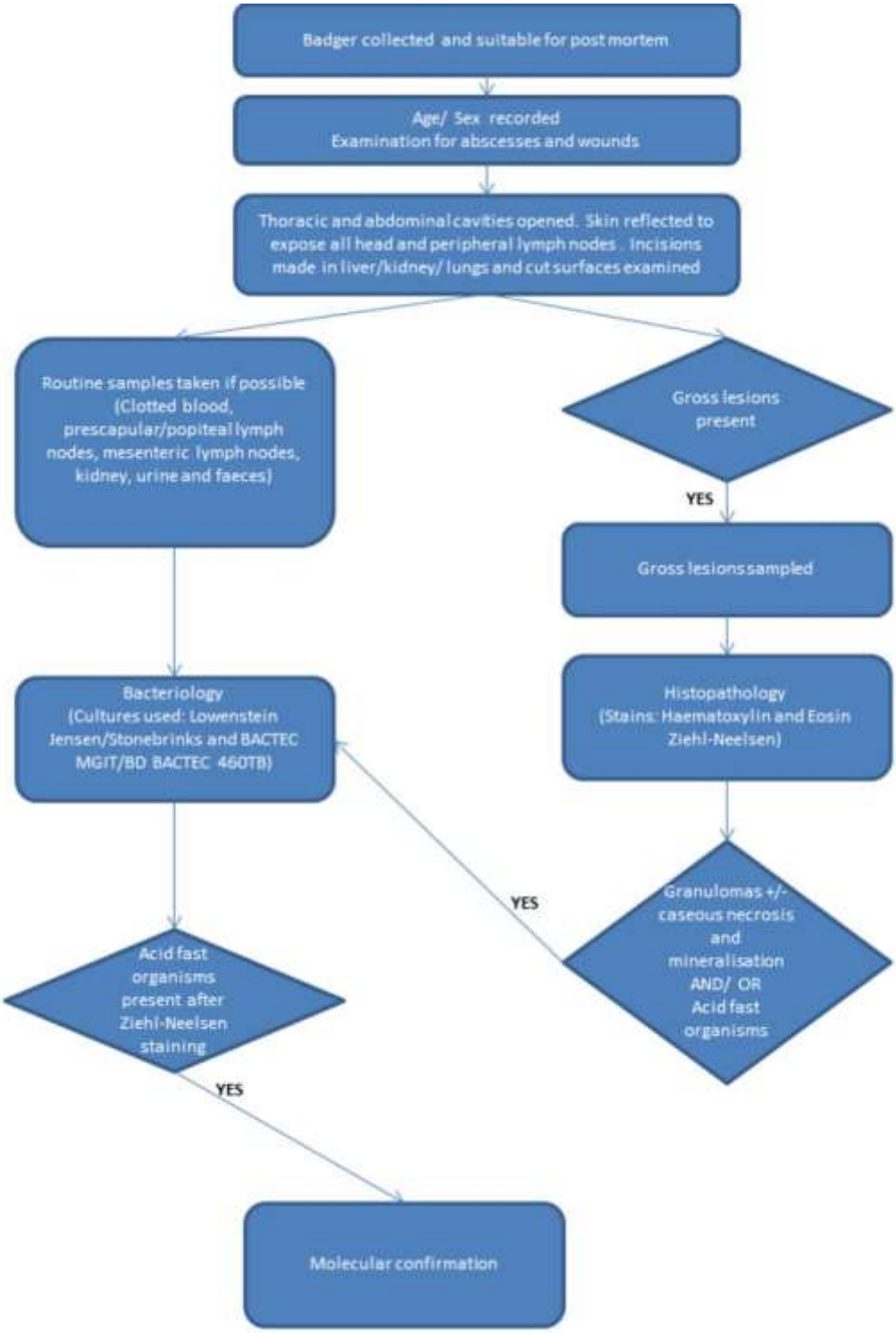

FIG 1: Diagnostic process for badgers submitted for postmortem examination.

start of the period. All lymph node pools were collected, not incised, and were subjected to bacteriological culture. Non-lymph node samples were individually cultured if gross lesions were present. All culture-positive non-visible lesions were examined histologically. Suspect

lesions were fixed in 10 per cent buffered formalin and embedded in paraffin wax blocks. Five-micron thick sections were stained using haematoxylin and eosin and Ziehl-Neelsen methods and examined by histopathology. Lesions showing histological evidence of tuberculosis (ie, lesions characteristic of tuberculosis (granulomas ±caseous necrosis and mineralisation) and/or acid-fast organisms) were submitted for bacteriological culture. Culture was carried out in accordance with the OIE Manual of Standards for Tests and Vaccines.[5] All samples were cultured using both solid and liquid media (Lowenstein-Jensen/Stonebrink and BACTEC MGIT/BD BACTEC 460TB) except for faeces and urine, which were cultured using BACTEC MGIT/BD BACTEC 460TB only. Any cultures showing acid-fast organisms after Ziehl-Neelsen staining were sent for molecular confirmation. Confirmed *M bovis* isolates were subjected to molecular typing by the multilocus variable number of tandem repeat analysis (see Skuce *et al*[6]). *M bovis* was confirmed initially using Gen-Probe TB complex DNA probe test (Gen-Probe, San Diego, CA, USA) and more recently by identifying the *M bovis*-specific spoligotype signature.[7, 8] BD BACTEC MGIT 960 replaced the BD BACTEC 460TB during the study period. Internal laboratory validation showed no significant difference in performance (S.A.J. Strain, unpublished data). The case definition was a badger from which *M bovis* was isolated and molecularly confirmed from at least one of its samples.

TABLE 1: Sampling frequency of various sites from badgers suitable for postmortem examination

Sample site	Badgers sampled (n)	Percentage of badgers sampled
Kidney	1083	98.3
Lymph node pools	1056	95.8
Faeces	1041	94.5
Clotted blood	587	53.3
Urine	358	32.5
Abscess/wounds	58	5.3
Lung	16	1.5
Liver	10	0.9
Tissue was not identified	6	0.5
Spleen	2	0.2

Between December 9, 1998 and December 12, 2011, 1104 badgers were collected. Eighteen were excluded due to missing data (4 badgers had missing XY coordinates, 4 badgers were tagged incorrectly at collection while 10 had no or incomplete laboratory results available). The prevalence of *M bovis* was 15.3 per cent (95 per cent CI 13.1 to 17.5 per cent,

n=166/1086). Excluding 1998, the median number of badgers collected per year was 78 (ranging from 20 in 2001 to 136 in 2011). No statistically significant differences in the annual prevalence of *M bovis* were found (Fig 2).

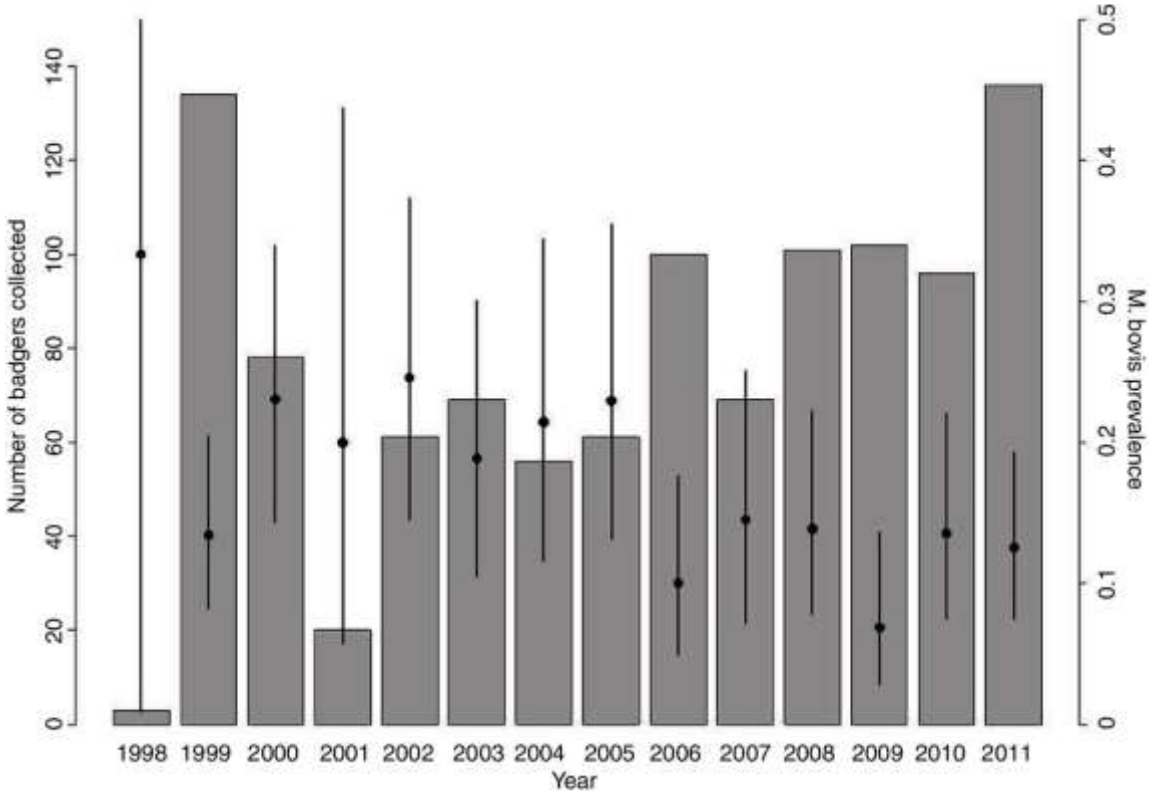

FIG 2: Number of badgers collected (bars) and annual *Mycobacterium bovis* prevalence (with 95 per cent binomial approximate confidence intervals; dots and lines).

Data on non-collected badgers were not routinely entered in the database until 2011. During this year, 136 (64 per cent) animals were collected of the 213 badgers reported. This figure is comparable to the 63 per cent of reported badgers collected in a similar study in Wales.[9] The reasons recorded for non-collection were 'Not located' (n=35, 45 per cent), 'Too damaged' (n=20, 26 per cent), 'Decomposed' (n=20, 26 per cent) and 'Too dangerous to collect' (n=2, 2.6 per cent).

Monthly peaks in badger collections were seen in February to March and again in September and October. There was no significant association between season and *M bovis* status (chi-squared P=0.461) or month and *M bovis* status (chi-squared P=0.23).

Of the badgers where sex was recorded, 47 per cent (n=438/932) were female and 53 per cent (n=494/932) were male. Male badgers were 1.59 times more likely to be *M bovis*-positive compared with female badgers (odds ratio (OR)=1.59; 95 per cent CI 1.08 to 2.35). There was no significant difference in weight between *M bovis*-positive and *M bovis*-negative badgers (positive mean=9.24 kg, negative mean=9.29 kg, *t* test P=0.89). Badgers found during the winter months (December to February) were 54 per cent more likely to be male than female (OR=1.54; 95 per cent CI 1.15 to 2.07) than in any other period during the year. There was a seasonal trend in weight, with lower weights being recorded in spring and summer (Kruskal-Wallis test P=0.002).

The most frequently sampled sites were the kidneys and lymph nodes, with lymph nodes taken from 95 per cent of badgers (Table 1). A mean of 4.9 sites per badger (sd=0.9) were sampled for bacteriological culture, with 16 badgers having no sites sampled for culture (1.5 per cent). There was no statistically significant difference in the mean number of sites sampled between *M bovis*-positive and *M bovis*-negative badgers (positive=5.05, negative 4.9, *t* test P=0.06). However, badgers that had more than five sites sampled were more likely to be *M bovis*-positive than those sampled five times or less (≤5 sites sampled OR=1, >5 sites sampled OR=1.91; 95 per cent CI 1.31 to 2.78). This reflects that sampling other than from kidneys, lymph node pools, faeces and urine was based on the presence of visible lesions. The objective of Table 2 was to examine whether certain regions were more likely to have positive samples than other sites. Therefore, the results used for Table 2 were restricted to those badgers sampled more than five times. Samples from the thorax were more likely to be positive compared with other sites (Table 2). For badgers culture-positive for *M bovis*, 9 per cent had positive urine samples, 14 per cent had positive faecal samples and 91 per cent had positive thoracic samples.

TABLE 2: Culture results of postmortem examination of badgers for *Mycobacterium bovis* where ≥4 sites were sampled overall (Odd ratios for *M bovis* being isolated from samples by anatomical region)

Region	Sites sampled if possible	Proportion of *M bovis*-positive (number of positive samples/number of samples collected)	Odds ratio (95% confidence interval)
Abdomen	Kidney, liver, mesenteric lymph node, spleen	0.05 (102/2022)	1
Carcase	Prescapular and popliteal pool	0.09 (76/831)	1.89 (1.37 to 2.61)
Head	Masseter muscle, retropharyngeal lymph node, submandibular lymph node, tonsil	0.17 (1/6)	3.76 (0.08 to 34.02)
Thorax	Lung, mediastinal lymph node	0.62 (8/13)	29.94 (8.47 to 118.71)
Other	Abscess swab, faeces, other lymph nodes, muscle, other lesions, urine	0.05 (114/2341)	0.96 (0.73 to 1.28)

Samples were taken if the tissue was not overly damaged.

Nearest neighbour analysis examined whether pairs of badgers associated spatially and temporally shared the same infection status (within 12 months of collection). The Euclidean distances in metres between each badger and its nearest positive and negative neighbouring badgers found in the preceding or subsequent 12 months were measured. The ratios between the distance to the nearest positive and negative neighbour for each badger were then calculated to overcome any biases due to differing badger densities.[10] Positive badgers were closer to other positives than they were to negative badgers—Positive badgers 2.40 (sd=2.36), negative badger=3.41 (sd=5.39); Mann-Whitney U test P=0.02[4].

The odds of a badger being collected relative to the estimated badger population[4] were calculated to determine if the survey was spatially biased (Table 3). In addition, the odds that a collected badger was *M bovis*-positive were also calculated for each county. The

collection of RTA badgers showed a spatial bias towards County Down. Badgers collected from County Fermanagh were more likely to be positive than those collected from other counties. These findings are likely to reflect a spatial bias within the survey.

TABLE 3. Number of badgers collected per county relative to the estimated badger population (OR).

County	Badgers positive (n)	Badgers collected (n)	Estimated badger population*	OR of being an RTA in the survey (95 per cent CI)	OR of being *Mycobacterium bovis*-positive (95 per cent CI)
Antrim	27	193	5800	0.75 (0.63 to 0.89)	1 (0.6 to 1.62)
Armagh	19	94	4500	0.46 (0.37 to 0.58)	1.56 (0.86 to 2.73)
Derry	14	135	4000	0.76 (0.62 to 0.92)	0.71 (0.37 to 1.29)
Down	58	414	9400	1	1
Fermanagh	14	54	3800	0.31 (0.23 to 0.41)	2.15 (1.07 to 4.13)
Tyrone	34	196	6500	0.68 (0.57 to 0.8)	1.29 (0.8 to 2.03)

*Taken from Reid and others.[4]
RTA, road traffic accident.

Sixty per cent of badgers were reported by departmental or associated government staff, 24 per cent by herd keepers, 11 per cent by members of the public, 4 per cent by the police and 1 per cent by private veterinary surgeons. Government staff, herd keepers and private veterinary surgeons were all more likely to report positive badgers than negative badgers: members of the public OR=1 (reference), staff OR=2.21 (95 per cent CI 1.19 to 4.43), herd keepers OR=2.26 (95 per cent CI 1.15 to 4.73), police=2.13 (95 per cent CI 0.77 to 5.73) and private veterinary surgeons OR=6.13 (95 per cent CI 1.34 to 26.47). We evaluated whether the local tuberculosis cattle herd prevalence was associated with the likelihood of reporting for each reporter type. Data on cattle were extracted from the Animal and Public Health Information System.[11] For each 5-km zone, the number of *M bovis*-positive unique herds (defined as having one or more tuberculosis reactors (defined as positive to the single intradermal comparative cervical tuberculin test) for 12 months preceding and 12 months following the date the badger was collected) was calculated, as well as the number of unique herds tested during the time period. The median *M bovis* herd prevalence between reporter types showed significant differences (Kruskal-Wallis chi-squared statistic=25.5, P<0.001), with herd keepers more likely to report badgers in areas with higher *M bovis* herd prevalence than other reporter types (Fig 3).

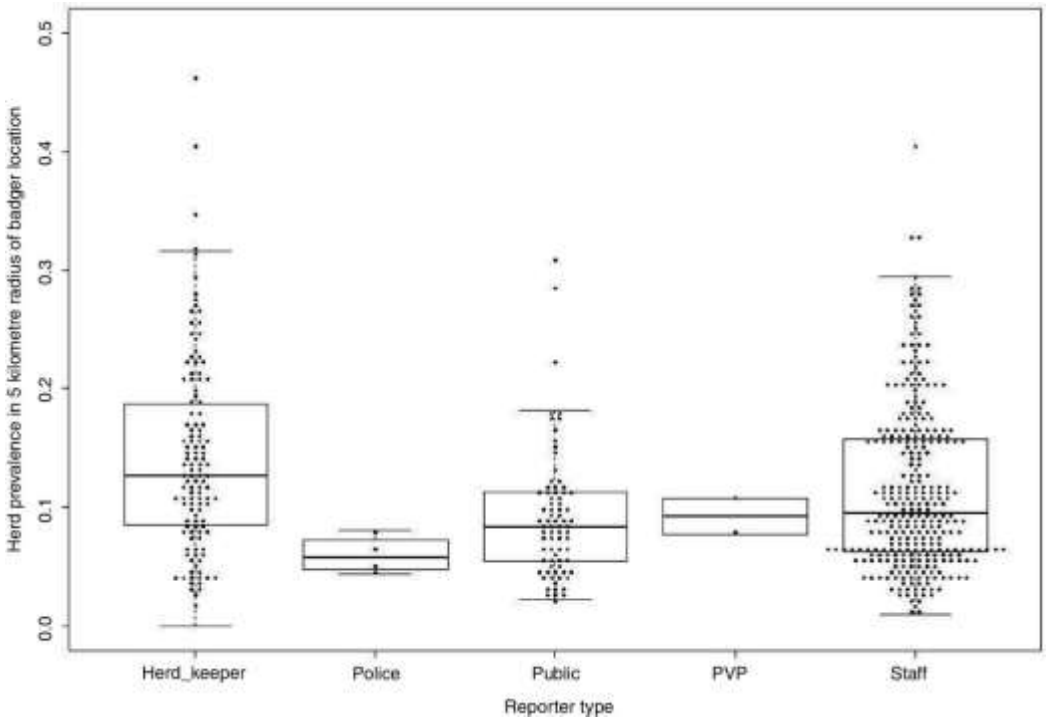

FIG 3: Cattle herd prevalence within a 5-km radius of location of the badger carcases in the preceding and following 12 months after collection. PVP, private veterinary practitioner.

There are a number of limitations to this survey. RTAs account for the largest cause of recorded deaths of badgers,[12, 13] but the badgers involved in these RTAs are unlikely to be representative of the underlying badger population; for example, these animals are more likely to be young male badgers. Additionally, reporting bias may have led to collections being more likely in certain geographical areas, for example, the over-representation of County Down (Table 2). Herd keepers may have been more motivated to report badger carcases if they have had a recent bTB herd breakdown, leading to a spatiotemporal bias. The results showed that badgers collected through reports from herd keepers were more likely to come from areas with a higher bTB herd prevalence than reports from members of the public, consistent with earlier studies in Northern Ireland.[14] The decision to collect a carcase was another possible source of bias. The reasons behind non-collection, as previously described, were unlikely to differ between infection status, and therefore it was probably not a significant source of bias.

Previous estimates from RTA badger surveys of the prevalence of *M bovis* from the British Isles are similar to our prevalence estimates (8.2–27.2 per cent in England and Wales[9,15]; 10–14 per cent in Ireland[16]). However, the prevalence is likely to be an underestimate given the low level of thoracic sampling undertaken, the reliance on gross pathology for sampling sites other than lymph nodes,[17] the well-documented limited sensitivity of bacterial culture/postmortem methods,[18] the variability of the quality and bacterial contamination of the carcases, and the potentially unrepresentative nature of the sample. In particular, the reliance of the study's postmortem procedure on gross pathology is likely to have significantly underestimated the proportion of *M bovis*-infected badgers by failing to detect non-visibly lesioned animals (see Corner *et al*[18]). Previous studies have demonstrated that the majority of infected badgers had no visible gross lesions.[19] Enhanced postmortem examination and culture in trapping studies has been shown to increase the diagnostic

sensitivity and lead to a threefold increase in prevalence.[17] However, it may not be feasible to consistently use it in RTA study designs, where the quality of the carcases is highly variable.

In agreement with published work,[9, 17] our results imply that excretion of *M bovis* by badgers is more likely to be via respiratory route rather than gastrointestinal or urinary tracts, and increasing the number of samples taken raises the odds of finding *M bovis* in a carcase. There was evidence that *M bovis*-infected badgers clustered in both time and space. The results of the survey have guided decisions for cattle bTB control at the local and national level, for example, local herd breakdown investigations and biosecurity advice,[2, 20] and have been used in the design of wildlife interventions and research.[21–23]

Despite the limitations, RTA surveys, compared with other field methods, represent a relatively inexpensive and non-invasive method to estimate the prevalence of tuberculosis in badgers.

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REFERENCES

1. ANON. Northern Ireland Assembly Committee for Agriculture and Rural Development Official Report (Hansard) Policy and Legislation 7 June 2011. 2011; http://www.niassembly.gov.uk/record/committees2011/Agriculture/110607Policy&Legislation.htm (accessed 8 Aug 2011).

2. Allen AR, Skuce RA, McDowell SWJ. *Bovine TB (TB): a review of badger-to-cattle transmission*, 2011. http://www.dardni.gov.uk/afbi-literature-review-tb-review-badger-to-cattle-transmission.pdf (accessed 8 Aug 2011).

3. ANON. *The agricultural census in Northern Ireland:* DAERA Policy and Economics Division, 2016:88.

4. Reid N, Etherington TR, Wilson GJ, et al. Monitoring and population estimation of the European badger *meles meles* in Northern Ireland. *Wildlife Biol* 2012;18:46–57. doi:10.2981/11-016

5. *OIE. Manual of diagnostic tests and vaccines for terrestrial animals 2016*, 2016. http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/ (accessed 8 Aug 2016).

6. Skuce RA, Mallon TR, McCormick CM, et al. *Mycobacterium bovis* genotypes in Northern Ireland: herd-level surveillance (2003 to 2008). *Vet Rec* 2010;167:684–9. doi:10.1136/vr.c5108

7. Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of *mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol* 1997;35:907–14.

8. Streicher EM, Victor TC, van der Spuy G, et al. Spoligotype signatures in the Mycobacterium tuberculosis complex. *J Clin Microbiol 2007;45:237–40.* Erratum in:J Clin Microbiol 45:3149. doi:10.1128/JCM.01429-06

9. Goodchild AV, Watkins GH, Sayers AR, et al. Geographical association between the genotype of bovine tuberculosis in found dead badgers and in cattle herds. *Vet Rec* 2012;170:259. doi:10.1136/vr.100193

10. Woodroffe R, Donnelly CA, Johnston WT, et al. Spatial association of *Mycobacterium bovis* infection in cattle and badgers *Meles meles. J Appl Ecol* 2005;42:852–62. doi:10.1111/j.1365-2664.2005.01081.x

11. Houston R. A computerised database system for bovine traceability. *Rev Sci Tech* 2001;20:652–61. doi:10.20506/rst.20.2.1293

12. Clarke GP, White PCL, Harris S. Effects of roads on badger *Meles meles* populations in south-west England. *Biol Conserv* 1998;86:117–24. doi:10.1016/S0006-3207(98)00018-4

13. Davies JM, Roper TJ, Shepherdson DJ. Seasonal distribution of road kills in the European badger *(Meles meles). J Zool* 1987;211:525–9. doi:10.1111/j.1469-7998.1987.tb01550.x

14. Menzies FD, Abernethy DA, Stringer LA, et al. A comparison of badger activity in two areas of high and low bovine tuberculosis incidence of Northern Ireland. *Vet Microbiol* 2011;151:112–9. doi:10.1016/j.vetmic.2011.02.033

15. ISG. *Bovine TB: The scientific evidence. Final report of the independent scientific group on cattle tuberculosis*. *London*: *DEFRA, 2007*.

16. O'Boyle I. *Tuberculosis investigation unit, selected papers from 1997 to 2002:* University College Dublin, 2002.

17. Murphy D, Gormley E, Costello E, et al. The prevalence and distribution of *Mycobacterium bovis* infection in European badgers *(Meles meles)* as determined by enhanced post mortem examination and bacteriological culture. *Res Vet Sci* 2010;88:1–5. doi:10.1016/j.rvsc.2009.05.020

18. Corner LA, Murphy D, Gormley E. *Mycobacterium bovis* infection in the Eurasian badger *(Meles meles):* the disease, pathogenesis, epidemiology and control. *J Comp Pathol* 2011;144:1–24. doi:10.1016/j.jcpa.2010.10.003

19. Gallagher J, Clifton-Hadley RS. Tuberculosis in badgers; a review of the disease and its significance for other animals. *Res Vet Sci* 2000;69:203–17. doi:10.1053/rvsc.2000.0422

20. Abernethy DA, Denny GO, Menzies FD, et al. The Northern Ireland programme for the control anderadication of *Mycobacterium bovis*. *Vet Microbiol* 2006;112:231–7. doi:10.1016/j.vetmic.2005.11.023

21. Biek R, O'Hare A, Wright D, et al. Whole genome sequencing reveals local transmission patterns of *Mycobacterium bovis* in sympatric cattle and badger populations. *PLoS Pathog* 2012;8:e1003008. doi:10.1371/journal.ppat.1003008

22. *DAERA. TVR wildlife intervention research project – year 1 report (2014),* 2015. www.daera-ni.gov.uk/publications/tvr-wildlife-intervention-research-project-year-1-report-2014 *(*accessed 1 Jul 2016*).*

23.Trewby H, Wright D, Breadon EL, et al. *U*se of bacterial whole-genome sequencing to investigate local persistence and spread in bovine tuberculosis. *Epidemics* 2016*;*14*:*26–35. doi:10.1016/j.epidem.2015.08.003