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

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Abstract

A road traffic accident survey began in 1998 in Northern Ireland to describe the occurrence of *Mycobacterium bovis* within the badger population. Between 1998 and 2011, 1104 badgers were collected with an overall prevalence of *M. bovis* of 15.3% (95% CI 13.1-17.5%). Male badgers were 1.6 times more likely to be *M. bovis* positive than females (Odds ratio =1.59; 95%CI 1.08-2.35). Badgers positive for *M. bovis* appeared to cluster together in space and time. Despite limitations, road traffic accident surveys represent a relatively inexpensive and non invasive method to estimate badger tuberculosis prevalence when compared to other methods in the field.

Keywords: Badger; Surveillance ;Tuberculosis

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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 for 2010/2011 (Anon 2011). There is evidence that badgers play a role in the maintenance and spread of *Mycobacterium bovis* to cattle (as reviewed by Allen and others 2011). Northern Ireland is a small country (13,843 km^2) whose agricultural land is dominated by grass production that supports 1.6 million cattle among 20,000 farms (Anon, 2016). The estimated badger population of 34,100 (95% confidence level (CI) 26,200-42,000) is widespread and contained within 7,600 social groups (95% CI 6,200 – 9,000) (Reid and others 2011). 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 dedicated collection vehicle were used for collection of badger carcasses for the survey. All reports of badger carcasses found on roads were followed up where possible. To minimise reporting bias, the reporting of carcasses was initially limited to Department of Agriculture, Environment and Rural Affairs (DAERA) employees and certain other public sector organisations but it was later widened to include herd keepers and members of the public. Any carcasses found where the cause of death was suspected to be non-accidental were reported to the local police wildlife officer and excluded from the study. Only carcasses deemed suitable for post-mortem were taken to the nearer of two veterinary diagnostic laboratories (located in Belfast or Omagh).

Submitted carcasses were placed in a Class I fume cabinet or on a down ventilated bench where a detailed post-mortem examination was normally carried out within 24 hours of

submission (see Figure 1). The sex and approximate age of the badger was recorded and the carcass 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. 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, lungs and the cut surfaces 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 start of the period. All lymph node pools 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% 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 showinghistological evidence of tuberculosis (i.e. 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 (OIE 2016). All samples were cultured using both solid and liquid media (Lowenstein Jensen/Stonebrinks and Bactec MGIT/ BD BACTEC 460TB) except 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 multi-locus VNTR analysis (Variable Number of Tandem Repeats) (see Skuce and others 2010). *M. bovis* was confirmed initially using GenProbe TB complex DNA probe test (Gen-Probe, San Diego, California) and more

recently by identifying the *M. bovis*-specific spoligotype signature (Kamerbeek and others 1997, Streicher and others 2007). 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* isolated and molecularly confirmed from at least one of its samples.

Between 9 December 1998 and 12 December 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% (95% CI 13.1-17.5%, *n*=166/1086). Excluding 1998, the median number of badgers collected per year was 78 (range 20 in 2001 to 136 in 2011). No statistically significant differences in the annual prevalence of *M. bovis* were found. (Figure 2).

Data on non collected badgers were not routinely entered on to the database until 2011. In this year, 136 (64%) animals were collected of the 213 badgers reported. This figure is similar to the 63% of reported badgers collected in a similar study in Wales (Goodchild and others 2012). Reasons recorded for non collection were "Not located" (n=35, 45%), "Too damaged" (n=20, 26%), "Decomposed" (n=20, 26%) and "Too dangerous to collect" (n=2, 2.6%).

Monthly peaks were seen in badger collections in February to March and again in September and October. There was no significant association between season and *M. bovis* status (χ^2 P=0.461) or month and *M. bovis* status (χ^2 P=0.23).

Of the badgers where the sex was recorded, 47% (*n*=438/932) were female and 53% (*n*=494/932) were male. Males were 1.59 times more likely to be *M. bovis* positive compared to females (odds ratio (OR)=1.59; 95%CI 1.08-2.35). There was no significant difference in weight between positive and negative badgers (positive mean= 9.24kg, negative mean= 9.29kg, *t* test *P*=0.89). Badgers found in the winter months (December through to February) were 54% more likely to be male than female (OR=1.54; 95% CI 1.15-2.07) than at 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% 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%). There was no statistically significant difference in the mean number of sites sampled between *M. bovis* positive and negative badgers (Positive =5.05, Negative 4.9, *t* test p=0.06). However, badgers that had more than 5 sites sampled were more likely to be *M. bovis* positive than those sampled 5 times or less (≤5 sites sampled OR= 1, >5 sites sampled OR=1.91; 95%CI 1.31-2.78). 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 5 times. Samples from the thorax were more likely to be positive compared to other sites (Table 2). For badgers culture positive for *M. bovis*, 9% had positive urine samples, 14% had positive faecal samples and 91% had positive thoracic samples.

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 twelve 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 (Woodroffe and others 2005). Positive badgers were closer to other positives than they were to negative badgers:- ratio between distance to nearest positive and negative badger :- Positive badgers 2.40 (SD= 2.36), Negative badger = 3.41 (SD=5.39), Mann Whitney U test P =0.02.

The odds of a badger being collected relative to the estimated badger population (taken from Reid and others, 2011) 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 in other counties. These findings are likely to reflect aspatial bias within the survey.

Sixty percent of badgers were reported by Departmental or associated government staff, 24% by herd keepers, 11% by members of the public, 4% by the police and 1% 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% CI 1.19-4.43), herd keepers OR= 2.26 (95% CI 1.15-

4.73), police= 2.13 (95% CI 0.77-5.73), and private veterinary surgeons OR= 6.13 (95% CI 1.34-26.47). We evaluated whether the local tuberculosis cattle herd prevalence was associated with the likelihood of reporting for each reporter type. Cattle data were extracted from the Animal and Public Health Information System (Houston 2001). For each five kilometre zone, the number of *M. bovis* positive unique herds (defined as having one or more tuberculosis reactors (defined as positives to thesingle 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 prevalences than other reporter types (Figure 3).

There are a number of limitations to this survey. Road traffic accidents account for the largest cause of recorded deaths of badgers (Clarke and others 1998; Davies and others 1987) but the badgers involved in these road traffic accidents are unlikely to be representative of the underlying badger population e.g. these animals are more likely to be young males. Additionally, reporting bias may have lead to collections being more likely in certain geographical areas e.g. the over-representation of County Down (Table 2). Herd keepers may have been more motivated to report badger carcasses if they have had a recent bTB herd breakdown leading to a spatio-temporal bias. The results showed that badgers collected through herd keeper reports 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 (Menzies and others 2011). The decision to collect a carcass 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 estimate (8.2-27.2% -England and Wales (ISG 2007; Goodchild and others 2012), 10-14% -Ireland (O'Boyle 2002)). 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 (see Murphy and others 2010), the well documented limited sensitivity of bacterial culture/ post mortem methods (Corner and others 2011), the variability of the quality and bacterial contamination of the carcasses and the potentially unrepresentative nature of the sample. In particular, the study post-mortem procedure's reliance 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 and others 2011). Previous studies have demonstrated that the majority of infected badgers had no visible gross lesions (Gallagher and Clifton-Hadley 2000). Enhanced post mortem examination and culture in trapping studies has been shown to increase the diagnostic sensitivity and lead to a three-fold increase in prevalence (Murphy and others 2010). However it may not be feasible to consistently be used in RTA study designs where the quality of the carcasses is highly variable.

In agreement with published work (Murphy and others 2010, Goodchild and others 2012), our results imply that excretion of *M. bovis* by badgers is more likely to be via the respiratory route rather than gastrointestinal or urinary tracts and increasing the number of samples taken raises the odds of finding *M. bovis* in a carcass. There was evidence that *M. bovis* infected badgers clustered in both time and space. The survey results have guided decisions for cattle

bTB control at the local and national level, e.g. local herd breakdown investigations and biosecurity advice (Abernethy and others 2006 , Allen and others 2011), and has been used in the design of wildlife interventions and research (Biek and others 2012, DAERA 2015 and Trewby and others 2016).

Despite the limitations, road traffic accident surveys represent a relatively inexpensive and non invasive method to estimate badger tuberculosis prevalence compared to other field methods.

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References

Abernethy, D. A., Denny, G. O., Menzies, F. D., McGuckian, P., Honhold, N., & Roberts, A. R. (2006). The Northern Ireland programme for the control and eradication of Mycobacterium bovis. *Veterinary Microbiology*, *112* (2), 231-237.

Allen, A. R., Skuce, R. A. & McDowell, S. W. J. (2011). Bovine TB (TB): a review of badger-to-cattle transmission. http://www.dardni.gov.uk/afbi-literature-review-tb-review-badger-to-cattle-transmission.pdf. Accessed August 8, 2011

ANON (2016) The Agricultural Census in Northern Ireland. DAERA Policy and Economics Division, Pg 88.

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

Biek, R., O'Hare, A., Wright, D., Mallon, T., McCormick, C., Orton, R. J. McDowell, S., Trewby, H., Skuce, R.A. & Kao, R. R. (2012). Whole genome sequencing reveals local transmission patterns of *Mycobacterium bovis* in sympatric cattle and badger populations. *PLoS Pathogens*, *8* (11), e1003008.

Clarke, G.P., White, P.C. & Harris, S., (1998) Effects of roads on badger *Meles meles* populations in south-west England. *Biological Conservation*, *86*(2), 117-124.

Corner, L. A. L., Murphy, D. & Gormley, E. (2011). *Mycobacterium bovis* Infection in the Eurasian Badger (*Meles meles*): the Disease, Pathogenesis, Epidemiology and Control. *Journal of Comparative Pathology*, *144* (1), 1-24.

DAERA (2015) TVR Wildlife Intervention Research Project – Year 1 Report (2014). www.daera-ni.gov.uk/publications/tvr-wildlife-intervention-research-project-year-1-report-2014. Accessed July 1, 2016

Davies, J.M., Roper, T.J. & Shepherdson, D.J., (1987) Seasonal distribution of road kills in the European badger (*Meles meles*). *Journal of Zoology*, *211*(3), 525-529.

Gallagher, J. & Clifton-Hadley, R. S. (2000). Tuberculosis in badgers; a review of the disease and its significance for other animals. *Research in Veterinary Science*, *69* (3), 203-217.

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. *Veterinary Record*, *170*, 259-259.

Houston, R. (2001) A computerised database system for bovine traceability. *Rev. Off. Int. Epizoot, 20*. 652

ISG (2007). Bovine TB: The Scientific Evidence. Final report of the Independent Scientific Group on cattle tuberculosis. London, DEFRA.

Kamerbeek, J., Schouls, L., Kolk, A., Van Agterveld, M., Van Soolingen, D., Kuijper, S., Bunshoten, A., Molhuisen, H., Shaw, R., Goyal, M. & Van Embden, J. (1997). Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *Journal of Clinical Microbiology*, *35* (4), 907-914.

Menzies, F.D., Abernethy, D.A., Stringer, L.A. and Jordan, C., (2011). A comparison of badger activity in two areas of high and low bovine tuberculosis incidence of Northern Ireland. *Veterinary Microbiology*, *151*(1), 12-119.

Murphy, D., Gormley, E., Costello, E., O'Meara, D. & Corner, L. A. L. (2010). The prevalence and distribution of *Mycobacterium bovis* infection in European badgers (*Meles meles*) as determined by enhanced post-mortem examination and bacteriological culture. *Research in Veterinary Science*, *88,* 1-5.

O'Boyle, I. (2002). Tuberculosis Investigation Unit, University College Dublin. Selected Papers from 1997 to 2002.

OIE (2016). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016. http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/ Accessed 8th August 2016

Reid, N., Etherington, T. R., Wilson, G. J., Montgomery, W. I. & McDonald, R. A. (2011). Monitoring and population estimation of the European badger *Meles meles* in Northern Ireland. *Wildlife Biology*, *18* (1), 46-57.

Skuce, R. A., Mallon, T. R., McCormick, C. M., McBride, S. H., Clarke, G., Thompson, A., Couzens, C., Gordon, A. W. & McDowell, S. W. J. (2010). *Mycobacterium bovis* genotypes in Northern Ireland: herd-level surveillance (2003 to 2008). *Veterinary Record*, *167* (18), 684-689.

Trewby, H., Wright, D., Breadon, E.L., Lycett, S.J., Mallon, T.R., McCormick, C., Johnson, P., Orton, R.J., Allen, A.R., Galbraith, J. & Herzyk, P., (2016). Use of bacterial whole-genome sequencing to investigate local persistence and spread in bovine tuberculosis. *Epidemics*, *14*, 26-35.

Streicher, E.M., Victor, T.C., van der Spuy, G., Sola, C., Rastogi, N., van Helden, P.D., Warren RM. (2007). Spoligotype signatures in the *Mycobacterium tuberculosis* complex. *Journal of Clinical Microbiology*. *45* (1), 237-40. Erratum in: *Journal of Clinical Microbiology*. *45* (9):3149.

Woodroffe, R., Donnelly, C. A., Johnston, W. T., Bourne, F. J., Cheeseman, C. L., Clifton-Hadley, R. S., Cox, D. R., Gettinby, G., Hewinson, R. G., Le Fevre, A. M., *et al.* (2005). Spatial association of *Mycobacterium bovis* infection in cattle and badgers *Meles meles*. *Journal of Applied Ecology*, *42*, 852-862.

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313 **Table 1**

314 Sampling frequency of various sites from badgers suitable for post mortem

Sample site	Number of badgers sampled	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

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317 **Table 2**

318 Culture results of badger post-mortem examination for *M. bovis* where ≥ 4 sites sampled

319 overall with odd ratios for *M. bovis* being isolated from samples by anatomical region.

320 (Samples were taken if the tissue was not overly damaged)

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Region	Sites sampled if possible	Proportion *M. bovis* positive (Number of samples positive / Number of samples collected)	Odds ratio (95%CI)
Abdomen	Kidney, liver, mesenteric lymph node, spleen	0.05 (102/2022)	1
Carcass	Prescapular & popliteal pool	0.09 (76/831)	1.89 (1.37-2.61)
Head	Masseter muscle, retropharygeal lymph node, submandibular lymph node, tonsil	0.17 (1/6)	3.76 (0.08-34.02)
Thorax	Lung, mediastinal lymph node	0.62 (8/13)	29.94 (8.47-118.71)

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Other	Abscess swab, faeces, other lymph nodes,muscle, other lesions, urine	0.05 (114/2341)	0.96 (0.73-1.28)

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Table 3

Number of badgers collected per county relative to the estimated badger population (OR = Odds ratio). *taken from Reid *and others.* (2012) OR= Odds ratio, 95%CI= 95% confidence interval

County	**No of badgers positive**	**No badgers collected**	**Estimated badger population***	**OR of being an RTA in the survey (95% CI)**	**OR of being *M. bovis* positive (95% CI)**
Antrim	27	193	5800	0.75(0.63-0.89)	1(0.6-1.62)
Armagh	19	94	4500	0.46(0.37-0.58)	1.56(0.86-2.73)
Derry	14	135	4000	0.76(0.62-0.92)	0.71(0.37-1.29)
Down	58	414	9400	1	1
Fermanagh	14	54	3800	0.31(0.23-0.41)	2.15(1.07-4.13)
Tyrone	34	196	6500	0.68(0.57-0.8)	1.29(0.8-2.03)

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Figure legends

Figure 1 Diagnostic process for badgers submitted for post mortem

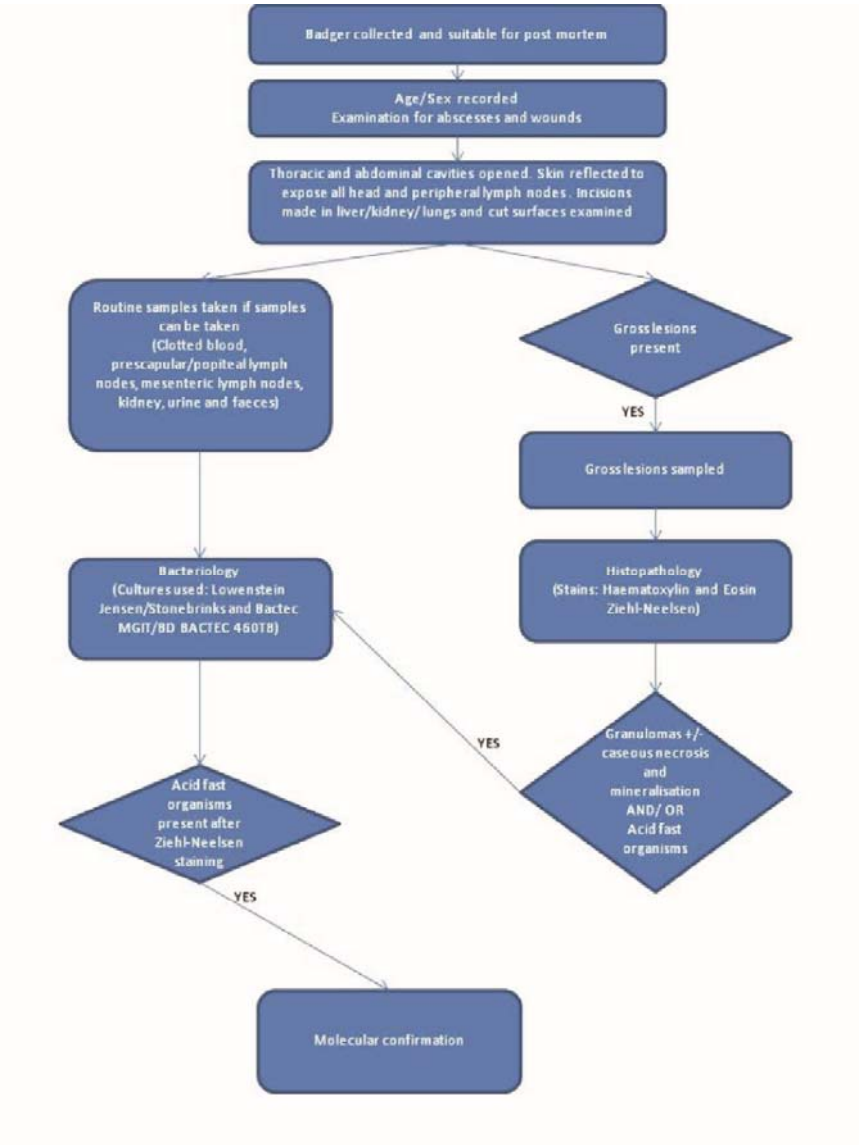

Figure 2 Number of badgers collected (bars) and annual *M. bovis* prevalence (with 95% binomial approximate confidence intervals; dots and lines)

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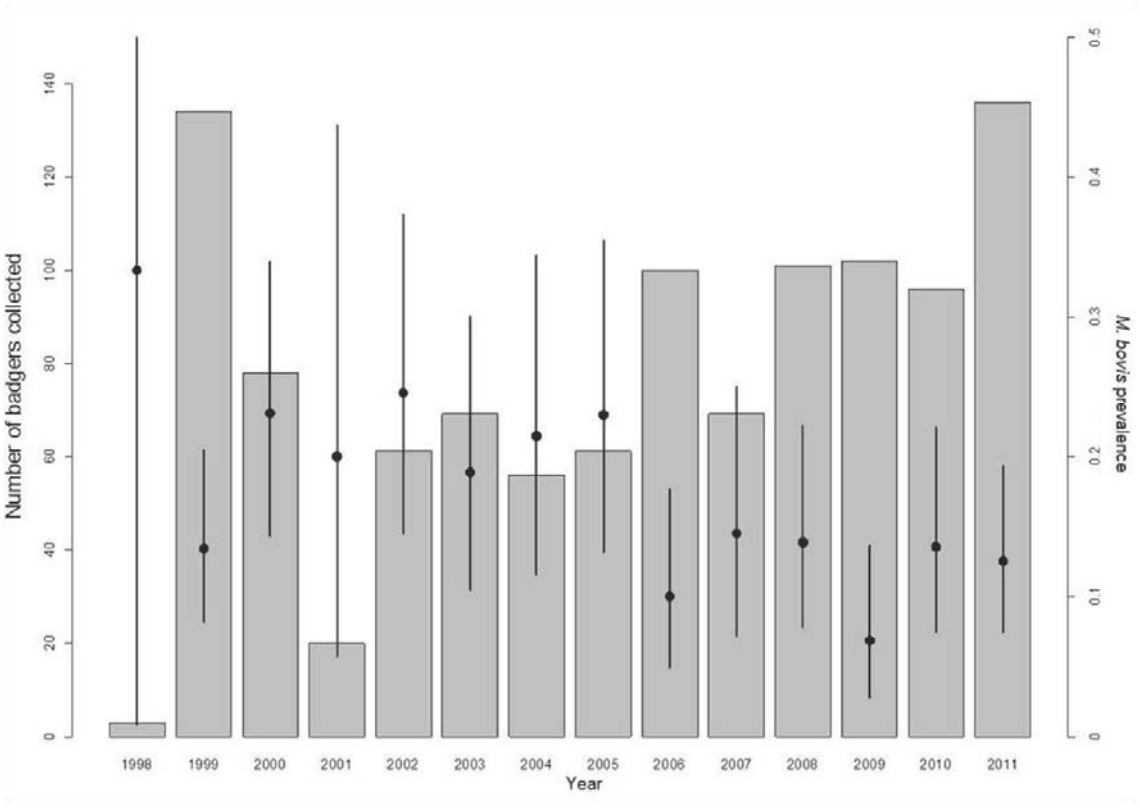

Figure 3 Cattle herd prevalence within a five kilometre radius of location of the badger carcass in the preceding and following 12 months after collection. (PVP= Private veterinary practitioner)

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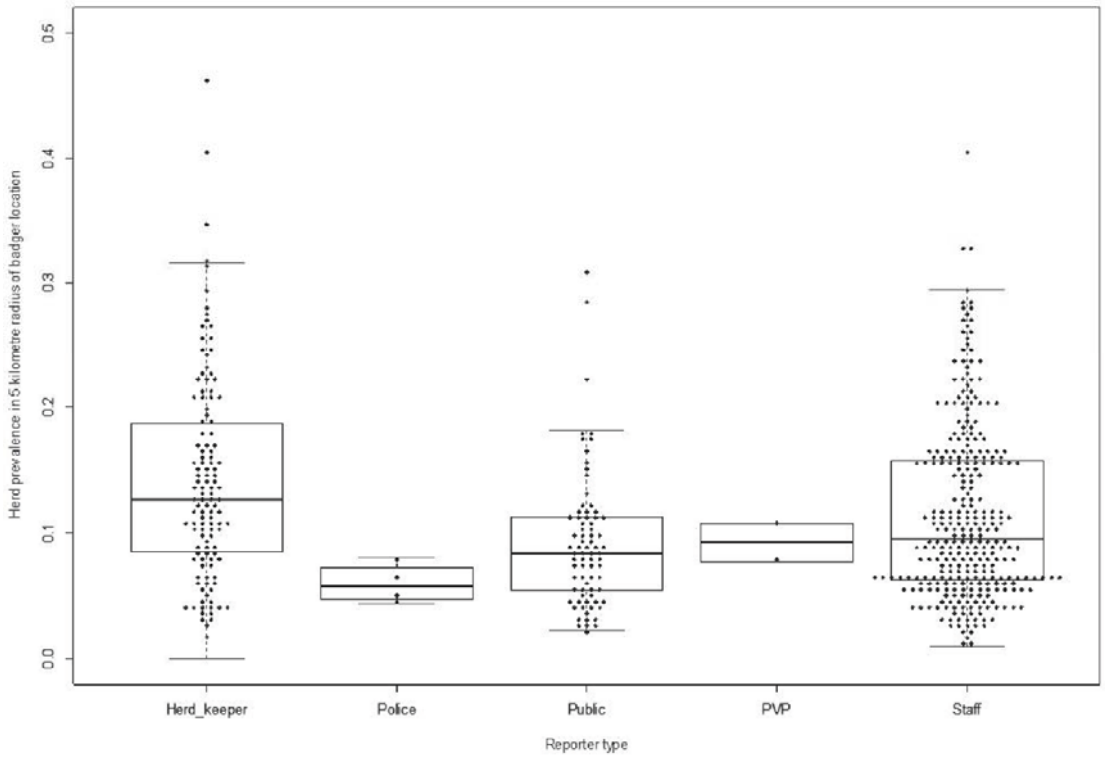

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