Bovine tuberculosis infection in wild mammals in the South-West region of England: A survey of prevalence and a semi-quantitative assessment of the relative risks to cattle

R.J. Delahay a,*, G.C. Smith a, A.M. Barlow b, N. Walker a, A. Harris c, R.S. Clifton-Hadley d, C.L. Cheeseman a

a Wildlife Disease Ecology Team, Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK
b Veterinary Laboratories Agency, Langford House, Langford, Somerset BS40 5DX, UK
c Microbiology Team, Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK
d Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB, UK

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Abstract

In the United Kingdom, badgers are implicated in the transmission of Mycobacterium bovis to cattle, but little information is available on the potential role of other wild mammals. This paper presents the results of the largest systematic UK survey of M. bovis infection in other wild mammals. Mammal carcasses (4715) from throughout the South-West region of England were subjected to a systematic post mortem examination, microbiological culture of tissues and spoligotyping of isolates. Infection was confirmed in fox, stoat, polecat, common shrew, yellow-necked mouse, wood mouse, field vole, grey squirrel, roe deer, red deer, fallow deer and muntjac. Prevalence in deer may have been underestimated because the majority were incomplete carcasses, which reduced the likelihood of detecting infection. Infected cases were found in Wiltshire, Somerset, Devon and Cornwall, Gloucestershire and Herefordshire. Lesions were found in a high proportion of spoligotype-positive fallow, red and roe deer, and a single fox, stoat and muntjac. M. bovis spoligotypes occurred in a similar frequency of occurrence to that in cattle and badgers. Data on prevalence, pathology, abundance and ecology of wild mammals was integrated in a semi-quantitative risk assessment of the likelihood of transmission to cattle relative to badgers. Although most species presented a relatively low risk, higher values and uncertainty associated with muntjac, roe, red and in particular fallow deer, suggest they require further investigation. The results suggest that deer should be considered as potential, although probably localised, sources of infection for cattle.

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Keywords: Mycobacterium bovis; Wildlife; Survey; Risk assessment

1. Introduction

Bovine tuberculosis (TB) caused by Mycobacterium bovis is a serious disease of domestic cattle, but has a wide range of other mammalian hosts, including humans. Despite the widespread success of cattle herd test and slaughter programmes, infection has persisted in some parts of the world where it has often been linked to a reservoir of infection in wild mammals.

In parts of Great Britain, particularly in South-West England, infection in cattle has steadily risen in recent years (Krebs, 1997; ISG, 2004). In the mid 1970s infected badgers (Meles meles) were detected in the vicinity of farms suffering from persistent breakdowns (Muirhead et al., 1974). Initial MAFF (Ministry of Agriculture, Fisheries and Food) investigations indicated that the prevalence of infection was higher in badgers than in other wild mammals (MAFF, 1976–1980; Little et al., 1982b). Since then

* Corresponding author. Tel.: +44 1904 462000; fax: +44 1904 462111. E-mail address: r.delahay@csl.gov.uk (R.J. Delahay).
the badger has been implicated in the transmission of infection to cattle and is widely believed to be the main wildlife reservoir of *M. bovis* in the United Kingdom. Although experimental studies in captivity indicate that transmission from badgers to cattle is possible (Little et al., 1982a), evidence that wild badgers contribute significantly to TB in cattle remains inconclusive (Krebs, 1997; Griffin et al., 2005). In addition, only limited investigations have been undertaken in the UK in the past on the prevalence of *M. bovis* infection in other wild mammals.

During previous studies, infection has been detected in red deer (*Cervus elaphus*), sika deer (*Cervus nippon*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*), fox (*Vulpes vulpes*), mink (*Mustela vison*), brown rat (*Rattus norvegicus*), feral ferret (*Mustela furo*), mole (*Talpa europaea*), free-living domestic cats (*Felis catus*) and a field vole (*Microtus agrestis*) (Delahay et al., 2002). The collated data from previous work suggested that prevalence levels were generally low, although non-systematic sampling, variations in diagnostic methodology and small sample sizes may have biased some studies (Delahay et al., 2002). However, prevalence levels alone are unlikely to provide sufficient information to assess the potential risks of transmission to cattle, as this will also be partly determined by host ecology, and the pathology and epidemiology of the disease in each species.

The present paper describes the results of a study to collect data on the distribution, frequency and pathology of *M. bovis* infection in British wild mammals other than the badger. Surveillance was focused on areas of high cattle herd breakdown risk in South-West England so as to increase the probability of detecting species that could be important in the epidemiology of bovine TB in cattle. Wild mammal carcasses were subjected to a systematic post mortem investigation, microbiological culture and molecular typing of isolates. This project represents the most extensive and systematic survey for *M. bovis* infection in wild mammals in the UK to date. Results of these investigations were combined with ecological information in a semi-quantitative assessment of the potential relative risks to cattle posed by wildlife hosts.

2. Materials and methods

2.1. Carcass collection

The target area for collection of wild mammal carcasses was defined as the entire South-West region of England. However, sampling effort was maximised in and immediately around ‘cattle TB hotspots’ within the treatment areas selected for the randomised badger culling trial (RBCT) (ISG, 1999). This was to allow eventual comparison with data on the TB status of the local badger populations. In addition, sampling effort was also concentrated in the vicinity of Woodchester Park, Gloucestershire, which has been the location of a long-term study of *M. bovis* in a naturally infected, undisturbed badger population (see Cheeseman et al., 1988; Delahay et al., 2000).

Carcasses were collected from a variety of sources. A network of local contacts was established throughout South-West England, of people who would come into the possession of mammal carcasses during their normal working routine. This included gamekeepers, deer stalkers, foresters, farmers, pest controllers, scientific researchers, veterinarians, wildlife rescue centres, local authorities, conservation bodies, government agencies, the police and the general public. However, most small mammals were obtained under licence from English Nature, by capture in Longworth traps (Gurnell and Flowerdew, 1994) before humane dispatch.

Each collected carcass was given a unique reference number and the species, date of collection, location, source and cause of death were recorded. Carcasses were transported in a refrigerated van (0–4 °C) to the veterinary pathology laboratory where they were stored in a cold-room (4 °C). Post mortem examination was carried out immediately wherever possible, and carcasses were only frozen as a last resort.

2.2. Post mortem examination

A systematic and comprehensive post mortem procedure for detecting *M. bovis* infection was developed and is suggested as a standard procedure for screening wild mammals for *M. bovis* infection. The following is a brief summary.

During initial examination the body weight, age group (adult or juvenile) and sex of each carcass was determined. The distinction between adult and juvenile animals was based on a range of factors such as gonad development, body weight and degree of tooth wear.

Each carcass underwent a systematic internal examination for lesions. This included the outer surfaces and incised sections of the internal lymph nodes (mandibular, retropharyngeal, prescapular, axillary, mediastinal, tracheobronchial, hepatic, mesenteric, lumbar, internal iliac, external iliac, and popliteal) and selected organs (lungs, heart, kidneys, liver, spleen) although it was often not possible to identify peripheral lymph nodes in the smaller mammals. A broad definition of “suspicious lesion” was adopted and each suspicious lesion was cultured separately to increase the sensitivity of screening. Suspicious lesions included any imprecisely circumscribed lesion of any colour in thoracic and visceral organs but did not include creamy nodular lesions in the diaphragmatic lobes of roe deer that were presumed to be caused by Protostrongylid lungworms, following histological examination of initial cases.

For cases where no macroscopic lesions were present, a pool of tissues consisting of portions of retropharyngeal, tracheobronchial, mediastinal and mesenteric lymph nodes together with portions of lung and spleen was collected for mycobacterial culture. In cases where visible lesions were identified, a similar pool of tissue was taken, plus additional samples of tissues with lesions, which were cultured
separately. In addition, smears were prepared from suspect lesions for Ziehl–Neelson staining to identify acid-fast bacilli (Gallagher and Horwill, 1977).

2.3. Mycobacterial culture of tissue samples

Each tissue sample was homogenised, inoculated onto six 7H11 agar slopes and incubated for 6 weeks at 37 °C (Gallagher and Horwill, 1977). The identity of suspect *M. bovis* colonies was confirmed by spoligotyping (Aranaz et al., 1996; Kamerbeek et al., 1997). No case was considered as a ‘confirmed positive’ unless an *M. bovis* spoligotype was identified. Cases for which a positive culture result was obtained that could not be subsequently confirmed as *M. bovis* by spoligotyping, were referred to as ‘unconfirmed cases’.

2.4. Analyses and risk assessment

Estimates of the prevalence of infection and associated 95% confidence intervals were calculated according to the methods described by Armitage and Berry (1994). Hence, they were based on the assumption that the total population size is infinite, although in practice this approximation is reasonable for non-infinite populations provided the sample size is not too large a proportion of the total. Statistical tests were carried out using Genstat (GenStat for Windows 6th Edition, VSN International Ltd.). Where sample size was sufficiently large, logistic regression (Collett, 1991) was carried out to assess age and sex specific differences in prevalence of infection for given species. Both age and sex were fitted independently and significance was assessed by referring the associated change in model deviance to a $\chi^2$ distribution. Logistic regression was also used to assess whether in deer the probability of detecting infection was influenced by the type of sample received (i.e. complete vs. incomplete carcasses) whilst including deer species as a covariate.

In order to integrate information on prevalence, pathology, abundance and ecology of host species, a semi-quantitative risk assessment was carried out using Crystal Ball (a Monte Carlo add-on to Microsoft Excel: Decisioneering). This was used to calculate the potential risk of transmission to cattle from each species for which a positive result was confirmed, relative to that of the badger. For each species, including the badger, ranges of potential values were determined for disease prevalence, estimated extent of bacterial excretion, likelihood of contact with cattle, and approximate biomass. For each of these four parameters, one value was randomly chosen from the distribution, the product of these four values was calculated, and then normalised (i.e. the output risk value for each species was divided by the risk value of the badger, thus producing a relative risk value) so that the risk of transmission from the badger was unity. Ten thousand such iterations were performed for each species. The range for each parameter was determined as follows:

1. **Prevalence of infection.** A triangular distribution centred on the best estimate, with the minimum and maximum set to the 95% confidence interval for confirmed cases only (see Table 1).
2. **Bacterial excretion.** Three of the authors (RJD, RCH and AB), independently used their judgement to determine the relative level of bacterial excretion compared to the badger (i.e. 0, 0.25, 0.5, 0.75, 1.0, 1.25 or 1.5) per unit weight of tissue. Each of these values produced a triangular range, with the minimum of the range one value lower (absolute minimum zero) and the maximum one value higher (see Fig. 1 for examples). Where substantial uncertainty existed, two or more triangular distributions were combined and weighted to ensure each expert was given similar weight.
3. **Contact with cattle.** Three of the authors (RJD, RCH and CLC), independently used their judgement to determine the relative potential for contact with cattle using the same method described above.

**Biomass.** Biomass was the product of bodyweight and density, with values taken from published sources (see Appendix 1). Biomass was used, rather than density, since the bacterial excretion was assessed per unit weight of tissue. Both bodyweight and density were defined as triangular distributions centred on published estimates, with the minimum and maximum determined by the published range.

Since the product of these parameters is normalised relative to the badger, a value of 1.0 would represent a risk of *M. bovis* transmission from the host to cattle similar to that of the badger, assuming that both were present at the stated density. For example a risk of 1.0 for muntjac (*Muntiacus reevesi*) would only apply in areas where that species were present at moderate to high density. We present the median result of the iterations as the best estimate, and the inter-quartile range as a measure of uncertainty.

3. Results

3.1. Carcass collection

Post mortem examination and microbiological culture of tissue samples was carried out on 4714 wild mammal carcasses (see Table 1). A very small proportion of the deer carcasses was obtained from enclosed parkland populations (40 fallow and three sika deer). In addition, three roe deer, six fallow deer and one muntjac were submitted because they were suspected to be infected following cursory examination by a stalker. Of the submitted ‘suspects’, all but one roe deer and two fallow deer were confirmed positive on culture and spoligotyping. As these animals could not be considered as a random sample of free-living deer they were not included in prevalence calculations and were omitted from further analyses (except those relating to the descriptions of pathology).
Table 1
Estimates of the prevalence of *M. bovis* confirmed cases (and 95% confidence intervals) for wild mammals collected and examined during the study (omitting deer submitted because they were suspects and those from enclosed populations)

<table>
<thead>
<tr>
<th>Species</th>
<th>Number collected</th>
<th>Prevalence of culture positive cases confirmed by spoligotype</th>
<th>Prevalence</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td>Fox</td>
<td>756</td>
<td>732</td>
<td>24</td>
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<td>Otter</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>0.00</td>
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<tr>
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<td>51</td>
<td>51</td>
<td>0</td>
<td>0.00</td>
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<td>78</td>
<td>75</td>
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<td>3.85</td>
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<td>50</td>
<td>0</td>
<td>0.00</td>
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<td>23</td>
<td>1</td>
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<tr>
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<td>1</td>
<td>0</td>
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<td>83</td>
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<td>0.00</td>
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<td>157</td>
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<td>1.49</td>
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<td>317</td>
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<td>0.00</td>
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<td>9</td>
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<td>194</td>
<td>2</td>
<td>1.02</td>
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<td>3</td>
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<td>0.00</td>
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<tr>
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<td>482</td>
<td>22</td>
<td>4.37</td>
</tr>
<tr>
<td>Muntjac</td>
<td>58</td>
<td>55</td>
<td>3</td>
<td>5.17</td>
</tr>
<tr>
<td>Feral sheep</td>
<td>5</td>
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<td>7</td>
<td>7</td>
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<td>0.00</td>
</tr>
</tbody>
</table>

![Fig. 1](https://via.placeholder.com/150)

Fig. 1. Examples of the distributions for single and combined relative risks used for calculating the range of bacterial excretion and potential for contact with cattle. The example distributions are (a) 0, (b) 0.5, (c) 0 plus 0.25, (d) 0.25 plus 0.50 and (e) 1.0 to 1.5.
Mammal carcasses were obtained from a variety of sources, the majority being by-products of routine pest and game management. Hence, for some species such as roe deer, fallow deer and fox relatively large numbers were collected (Table 1). The majority of deer (84.8%) and foxes (77.4%) were shot by stalkers and gamekeepers as part of their routine operations, and most moles (86.7%), grey squirrels (Sciurus carolinensis) (92.6%) and brown rats (95.6%) were obtained as a result of pest management.

Inevitably sample sizes were substantially lower for the less abundant and protected species, such as polecats (Mustela putorius) and otters (Lutra lutra) (Table 1). A substantial proportion (27.4%) of mustelids (i.e. otter, mink, feral ferret, polecat, stoat, weasel (Mustela nivalis)) were obtained as a result of RTAs (road traffic accidents), although with the exception of otters, the majority (56.4%) were trapped by gamekeepers as part of their routine management practices. Small mammals (i.e. mice, voles, shrews) however were largely obtained by trapping (87.7%) specifically undertaken for the present project. For most species, including deer (11.6%), foxes (10.2%), small mammals (0.3%) and lagomorphs (9.1%), the proportion obtained from RTAs was relatively small. Veterinary surgeries and wildlife rescue centres provided carcasses of a variety of species, including the majority (62.3%) of hedgehogs.

3.2. Prevalence estimates

During post mortem examinations macroscopic lesions suggestive of M. bovis infection were detected in several species (n = 99 cases), but only 35.4% (n = 35) yielded positive culture results, three of which could not subsequently be spoligotyped. Suspect lesions that failed to produce isolates and culture isolates that could not be confirmed by spoligotyping were most frequently observed in deer (see Table 2). For example, 45% (n = 18) of culture positive roe deer could not be confirmed as M. bovis positive by spoligotyping.

M. bovis infection was detected by microbiological culture and confirmed by spoligotyping in 12 species (Table 1). However, only 72.4% of culture positive cases also produced an M. bovis spoligotype. Confirmed cases were identified in fox, stoat, polecat, common shrew (Sorex araneus), yellow-necked mouse (Apodemus flavicollis), wood mouse (Apodemus sylvaticus), field vole, grey squirrel, and red, fallow and muntjac deer (Table 1). All the culture positive, but spoligotype negative samples (i.e. unconfirmed cases) originated from species in which infection had been confirmed in other individuals (i.e. fox, grey squirrel and red, roe and fallow deer). One roe deer was infected with Mycobacterium kansasi.

Limited sample sizes meant that age and sex specific differences in the prevalence of infection could only be investigated for fox, fallow and roe deer (Table 3). The prevalence of infection was significantly higher in adults than juveniles for fallow (logistic regression, \( \chi^2 = 8.83, \text{df} = 1, \ P < 0.01 \)) and roe deer (logistic regression, \( \chi^2 = 4.7, \text{df} = 1, \ P < 0.05 \)). There were no age-related differences for foxes (logistic regression, \( P > 0.05 \)) although prevalence was significantly higher in females than males (logistic regression, \( \chi^2 = 5.49, \text{df} = 1, \ P < 0.05 \)). There were

<table>
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<tr>
<th>Species</th>
<th>No visible lesions culture positive</th>
<th>No visible lesions spoligotype-negative</th>
<th>Visible lesions culture negative</th>
<th>Visible lesions spoligotype-positive</th>
<th>Visible lesions culture positive</th>
<th>Visible lesions spoligotype-negative</th>
<th>Other mycobacteria</th>
<th>Total</th>
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<td>0</td>
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<td>1</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Muntjac</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>42</td>
<td>64</td>
<td>3</td>
<td>32</td>
<td>1</td>
<td>166</td>
<td></td>
</tr>
</tbody>
</table>

Only those cases with culture positive results confirmed by spoligotyping were considered as ‘confirmed positives’. Those species in which no suspect lesions were observed and all culture results were negative have been omitted.
no sex-related differences for fallow and roe deer (logistic regression, \( P \) always \( > 0.05 \)).

Except for wild boar and deer, the majority of submitted samples were whole carcasses, with only 0.6% of the 3008 samples being incomplete. The seven wild boar samples consisted of only two whole carcasses, the remainder being five heads, two of which were accompanied by assorted organs. For deer, complete carcasses only accounted for 244 (14.8%) of the 1646 samples received. The remainder were described as either ‘gralloch’, or combinations of ‘pluck’ and head. The term gralloch was used to describe all the parts removed to dress a carcass, including the head, ‘pluck’ (lungs and heart) and abdominal viscera (stomach, small and large intestine, liver and kidneys). As the tissues available for examination differed according to the type of sample submitted, a logistic regression was performed to determine whether the probability of detecting infection varied accordingly (with deer species included as a covariate).

There were significantly higher probabilities of detecting suspect lesions (\( \beta_{\text{gralloch}} = -1.16, \chi^2 = 11.3, \text{df} = 1, P < 0.01, n = 865 \)) and of isolating \( M. \text{bovis} \) by culture (\( \beta_{\text{gralloch}} = -1.39, \chi^2 = 9.4, \text{df} = 1, P < 0.001, n = 865 \)) from whole carcasses than from gralloch. The model predicted probabilities (with 95% confidence intervals) of a culture positive sample for carcass and gralloch of 5.6% (3.0–10.4%) and 1.4% (0.7–2.7%) respectively. Consequently, prevalence estimates for \( M. \text{bovis} \) infection in deer were significantly lower when based on samples of gralloch (\( P < 0.01 \)) than for whole carcasses. This effect was particularly pronounced in fallow deer where the sample prevalence of infection estimated on the basis of gralloch (3.2%) was substantially lower than for whole carcasses (19.4%).

3.3. Spatial distribution of infection

Carcass collection focused on the areas encompassing the triplets of the randomised badger culling trial, although because of the voluntary nature of supply and the limited abundance and distribution of some species, collection also took place elsewhere. The majority of confirmed positive \( M. \text{bovis} \) cases came from east Cornwall, Herefordshire and the Gloucestershire Cotswolds where sample sizes were largest. Sample size restrictions meant that prevalence estimates were only calculated for selected species for the Cotswolds area (Table 4) and these were generally higher than those for the entire South-West region as shown in

<table>
<thead>
<tr>
<th>Area</th>
<th>Fallow deer</th>
<th>Roe deer</th>
<th>Muntjac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exmoor (Devon)</td>
<td>30.4</td>
<td>0 (0–7.22)</td>
<td>–</td>
</tr>
<tr>
<td>Southern Gloucestershire</td>
<td>25.7</td>
<td>5.91</td>
<td>2.70</td>
</tr>
<tr>
<td>Western Gloucestershire</td>
<td>39.6</td>
<td>3.39</td>
<td>–</td>
</tr>
<tr>
<td>Mendips (Somerset)</td>
<td>62.7</td>
<td>–</td>
<td>0 (0–1.80)</td>
</tr>
</tbody>
</table>

### Table 1

In addition, logistic regression analysis detected a significant variation in prevalence estimates between triplet areas of the RBCT for fallow deer (change in deviance = 15.5, df = 4, \( P < 0.01 \)), but not for roe deer, red deer and foxes (\( P \) always \( > 0.1 \)). At a finer spatial scale it was possible to calculate prevalence estimates for fallow, roe and muntjac deer in four specific localities of less than 100 km\(^2\) (Table 5).

### 3.4. Pathology

Amongst the confirmed positive cases (including those deer submitted as suspects and those from enclosed populations) gross pathology was observed in one fox, one stoat, and the four species of deer (Table 6). In the fox and stoat gross lesions were observed in the mesenteric lymph nodes. In contrast visible lesions were common in confirmed positive deer, particularly fallow, and \( M. \text{bovis} \) was isolated from a variety of lymph nodes and organs. Infection in roe and fallow deer was most frequently asso-

### Table 2

Frequency and prevalence of \( M. \text{bovis} \) confirmed cases in fallow deer, roe deer and foxes by age and sex (for some carcasses age and/or sex data was not available)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Males</th>
<th>Females</th>
<th>Juveniles</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow deer</td>
<td>9/190 (4.7)</td>
<td>11/235 (4.7)</td>
<td>0/75 (0)</td>
<td>19/296 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Roe deer</td>
<td>3/395 (0.8)</td>
<td>6/343 (1.7)</td>
<td>0/178 (0)</td>
<td>8/527 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Fox</td>
<td>8/427 (1.9)</td>
<td>16/326 (4.9)</td>
<td>2/81 (2.5)</td>
<td>21/651 (3.2)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3

Frequency and prevalence of \( M. \text{bovis} \) cases (and 95% confidence intervals) in the Gloucestershire Cotswolds (approximately 1700 km\(^2\) area)

<table>
<thead>
<tr>
<th>Species</th>
<th>Prevalence</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bank vole</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Field vole</td>
<td>1.64</td>
<td>0.04</td>
</tr>
<tr>
<td>Common shrew</td>
<td>2.86</td>
<td>0.07</td>
</tr>
<tr>
<td>Wood mouse</td>
<td>0.40</td>
<td>0.01</td>
</tr>
<tr>
<td>Yellow-necked mouse</td>
<td>2.86</td>
<td>0.07</td>
</tr>
<tr>
<td>Brown rat</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grey squirrel</td>
<td>0.58</td>
<td>0.01</td>
</tr>
<tr>
<td>Mole</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mink</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stoat</td>
<td>5.56</td>
<td>0.14</td>
</tr>
<tr>
<td>Fox</td>
<td>4.52</td>
<td>2.55</td>
</tr>
<tr>
<td>Roe deer</td>
<td>2.60</td>
<td>1.05</td>
</tr>
<tr>
<td>Fallow deer</td>
<td>7.20</td>
<td>4.39</td>
</tr>
<tr>
<td>Muntjac</td>
<td>6.82</td>
<td>1.43</td>
</tr>
</tbody>
</table>

Only data for culture positive cases confirmed by spoligotyping was included.

### Table 4

Estimates of the prevalence of \( M. \text{bovis} \) cases and 95% confidence limits in parentheses and sample size below) for specific geographical areas of less than 100 km\(^2\)

<table>
<thead>
<tr>
<th>Area</th>
<th>Prevalence</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exmoor (Devon)</td>
<td>30.4</td>
<td>0 (0–7.22)</td>
</tr>
<tr>
<td>Southern Gloucestershire</td>
<td>25.7</td>
<td>5.91</td>
</tr>
<tr>
<td>Western Gloucestershire</td>
<td>39.6</td>
<td>3.39</td>
</tr>
<tr>
<td>Mendips (Somerset)</td>
<td>62.7</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table 5

Estimates of prevalence of \( M. \text{bovis} \) positive deer (95% confidence limits in parentheses and sample size below) for specific geographical areas of less than 100 km\(^2\)

<table>
<thead>
<tr>
<th>Area</th>
<th>Fallow deer</th>
<th>Roe deer</th>
<th>Muntjac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exmoor (Devon)</td>
<td>30.4</td>
<td>0 (0–7.22)</td>
<td>–</td>
</tr>
<tr>
<td>Southern Gloucestershire</td>
<td>25.7</td>
<td>5.91</td>
<td>2.70</td>
</tr>
<tr>
<td>Western Gloucestershire</td>
<td>39.6</td>
<td>3.39</td>
<td>–</td>
</tr>
<tr>
<td>Mendips (Somerset)</td>
<td>62.7</td>
<td>–</td>
<td>0 (0–1.80)</td>
</tr>
</tbody>
</table>
### Table 6
Summary of pathological findings in confirmed positive mammal carcasses

<table>
<thead>
<tr>
<th>Number of confirmed positives</th>
<th>Frequency (%) with gross visible lesions</th>
<th>Spoligotype (frequency)</th>
<th>Location of confirmed lesions (frequency)</th>
<th>Cases with evidence of generalised infection (frequency)</th>
<th>Summary of typical gross pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fox</td>
<td>24 (4.17)</td>
<td>9 (1), 10 (1), 11 (1), 17 (21)</td>
<td>Mes (1)</td>
<td>0</td>
<td>Enlarged Mes, white abscesses up to 0.5 mm diameter</td>
</tr>
<tr>
<td>Stoat</td>
<td>3 (33.33)</td>
<td>10 (1), 17 (2)</td>
<td>Mes (1)</td>
<td>0</td>
<td>Enlarged Mes</td>
</tr>
<tr>
<td>Polecat</td>
<td>1</td>
<td>17 (1)</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Common shrew</td>
<td>1</td>
<td>17 (1)</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Yellow-necked mouse</td>
<td>1</td>
<td>9 (1)</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Wood mouse</td>
<td>2</td>
<td>17 (2)</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Field vole</td>
<td>1</td>
<td>17 (1)</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grey squirrel</td>
<td>2</td>
<td>11 (1), 17 (1)</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Roe deer</td>
<td>12</td>
<td>9 (1), 17 (10), 74 (1)</td>
<td>RP (3), L (4), B (2), Mes (3), II (2), P (1), Sp (2), O (1)</td>
<td>5</td>
<td>Creamy lesions &lt;10 cm diameter Encapsulated abscesses and caseous lesions &lt;1 cm diameter Enlarged RP. Unconfirmed small foci lesions in Med and Li. In one case 5 cm diameter abscesses in ribs</td>
</tr>
<tr>
<td>Red deer</td>
<td>2</td>
<td>11 (2)</td>
<td>SM (1), RP (1), L (1), B (1), Med (1), Liv (1)</td>
<td>2</td>
<td>Caseous lesions throughout</td>
</tr>
<tr>
<td>Fallow deer</td>
<td>28</td>
<td>9 (1), 10 (9), 17 (18)</td>
<td>RP (2), L (7), Med (4), B (4), Mes (7), II (1), Liv (2), O (1)</td>
<td>10</td>
<td>Creamy abscesses Encapsulated abscesses and caseous lesions &lt;7 cm diameter Generalised TB typically involving extensive purulent caseous lesions throughout L, B, Med and RP Multiple abscesses (&lt;0.5 cm diameter) in L Abscess (4 cm diameter) in pre-scapular lymph node Unconfirmed lesions in B and Med</td>
</tr>
<tr>
<td>Muntjac</td>
<td>4</td>
<td>17 (4)</td>
<td>L (1), PS (1)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Where suspect lesions were confirmed to be infected with *M. bovis* by microbiological culture and subsequent spoligotyping their location is given (SM = sub-mandibular lymph nodes, RP = retropharyngeal lymph nodes, L = lungs, Med = mediastinal lymph nodes, B = bronchial lymph nodes, PS = pre-scapular lymph nodes, Mes = Mesenteric lymph nodes, II = Internal Iliac lymph nodes, P = popliteal lymph nodes, Liv = liver, Sp = spleen, O = other.)
associated with the lungs and associated lymph nodes. For example, of the 12 fallow deer with confirmed lesions identified in specific lymph tissues and organs, 10 exhibited involvement of the lungs and associated lymph nodes (i.e. retro-pharyngeal, bronchial and mediastinal). Evidence of generalised widespread infection was found in 41.6% of confirmed positive roe deer and 35.7% of confirmed positive fallow deer.

3.5. Spoligotypes

Five *M. bovis* spoligotypes were identified from culture isolates (Table 6). The most common was type 17, which was also relatively geographically widespread and occurred in all infected species except red deer (type 11 only) and the yellow-necked mouse (type 9). All spoligotypes were detected in Gloucestershire where the intensity of sampling resulted in most positive cases. Type 10 was detected in fallow deer, a fox and a stoat, in a relatively localised cluster in Gloucestershire. Type 9 was found in roe deer in Gloucestershire and Cornwall, and in a yellow-necked mouse in Gloucestershire. Type 11 was isolated from red deer and a fox in Somerset, and a grey squirrel in Gloucestershire. Type 74 was only isolated from a single roe deer in Gloucestershire. The spoligotypes detected in the present study in Gloucestershire were compared to a database of types for slaughtered reactor cattle and infected badgers (three were RTAs, two were found dead and 125 had been culled during statutory badger removal operations) identified in the county from 1988 to 2003 (Table 7), and exhibited a close resemblance in type and relative frequency.

Table 7

<table>
<thead>
<tr>
<th>Spoligotype</th>
<th>Cattle</th>
<th>Badgers</th>
<th>Other mammals (present study)</th>
<th>Expected value (cattle)</th>
<th>Expected value (badgers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>185</td>
<td>6</td>
<td>11</td>
<td>12.0</td>
<td>2.6</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td></td>
<td></td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>17</td>
<td>542</td>
<td>112</td>
<td>42</td>
<td>35.3</td>
<td>49.1</td>
</tr>
<tr>
<td>21</td>
<td>2</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>22</td>
<td>23</td>
<td></td>
<td></td>
<td>1.5</td>
<td>0.0</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>35</td>
<td>3</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>74</td>
<td>3</td>
<td>1</td>
<td></td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>75</td>
<td>1</td>
<td></td>
<td></td>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td>81</td>
<td>6</td>
<td></td>
<td></td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td>89</td>
<td>11</td>
<td>3</td>
<td>5.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Total</td>
<td>860</td>
<td>130</td>
<td>57</td>
<td>56</td>
<td>57</td>
</tr>
</tbody>
</table>

Values were from random selections of cattle (from cattle herd breakdowns) and badgers (from culled and RTA badgers) from Gloucestershire during 1988–2003 inclusive (cattle and badger data supplied by Dr Noel Smith, Veterinary Laboratories Agency). The frequency of each spoligotype expected in ‘other mammals’ is given based on the observed frequency in cattle and badgers respectively.

3.6. Semi-quantitative risk assessment

The biomass of each species was calculated by including known variability in weight and density (see Table 8 and Appendix 1). The prevalence, mean likelihood of bacterial excretion, mean likelihood of contact with cattle and biomass for each species found to have confirmed infection in the present study, are given in Table 9. Although the badger had the highest prevalence, likelihood of excretion and contact rate with cattle, it had only the seventh highest biomass. Roe and fallow deer were considered to be likely to excrete *M. bovis* in similar quantities to badgers. Several deer species appear to pose a significant risk to cattle, relative to the badger, and the risk from fallow deer may exceed that of the badger. The combined risk from all deer species was 3.0, and for all other species was less than 0.05. Only five mammal species had a range width greater than 1: field vole (2.5), red deer (33.4), fallow deer (26.1), roe deer (4.9) and muntjac (11.9). Given that the median and mean risk for the field vole were less than 10% of those for the badger, this assessment suggests the former cannot be considered as a significant risk to cattle. In contrast, as the median risk and the uncertainty for roe, red, fallow and muntjac deer was relatively high, these species merit further investigation.

A sensitivity analysis was performed for the four deer species by determining the percentage contribution to the variance of the risk relative to the badger, of each of the input parameters (Table 10). For all species, since the final risk value was divided by the badger’s risk value to normalise each iteration, variation in badger prevalence, density and weight will appear as a contributor to the overall relative risk value. Also for all species the contribution to the variance for the combined badger variables was relatively high, but these parameters are well described by available data, as is the prevalence of infection for the deer species. In contrast, the relatively large influence of estimates of bacterial excretion and contact rates with cattle were associated with high levels of uncertainty. In addition, the range of density estimates for red and particularly fallow deer also had a relatively high percentage contribution to the variance in the model output.

4. Discussion

The present study represents the largest systematic survey for *M. bovis* in wild mammals in the UK to date. Infection was identified in several wild mammal species, some of which have been reported as hosts in previous studies (i.e. field vole, fox, red deer, fallow deer, roe deer, muntjac; see Delahay et al., 2002) and some of which were new records for the UK (i.e. yellow-necked mouse, wood mouse, common shrew, grey squirrel, stoat, polecat).

Prevalence estimates from the present study differed from those calculated by Delahay et al. (2002) using data from previous MAFF investigations (MAFF, 1976–97). For example, the MAFF data suggested a lower prevalence...
Table 8
Data on the abundance, body weight, distribution and habitat preferences of wild mammals found to be infected with *M. bovis* in the present study (see Appendix 1 for sources of data)

<table>
<thead>
<tr>
<th>Species</th>
<th>Density estimate</th>
<th>Mean body weight (kg)</th>
<th>Current distribution in South West England</th>
<th>Favoured habitats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey squirrel</td>
<td>0.5–2.7/ha (Coniferous), 2.4/ha (Oak/Hazel), 5.2–9.8/ha (Oak), 2–8 (mixed)</td>
<td>0.550&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Widespread and common</td>
<td>Mostly mature deciduous woodland, but also coniferous woodland, hedges and parkland</td>
</tr>
<tr>
<td>Yellow-necked mouse</td>
<td>Usually &lt;10/ha but up to 50/ha (woodland).</td>
<td>0.028&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Restricted to ancient woodland, therefore localised</td>
<td>Ancient deciduous woodland in particular, also semi-natural woodland close to agricultural land, hedgerows and field margins</td>
</tr>
<tr>
<td>Wood mouse</td>
<td>Varies with habitat and season: Mixed deciduous woodland 1–40/ha (summer), 97–128/ha (young larch plantation) and 1–15/ha (mixed farmland).</td>
<td>0.020&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Widespread and common</td>
<td>Highly adaptable, found in most habitats if not too wet</td>
</tr>
<tr>
<td>Field vole</td>
<td>Approximately 100–300/ha (grassland), 3–10/ha (woodland).</td>
<td>0.034&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Widespread but patchy</td>
<td>Rough ungrazed grassland. Common in meadows, field margins and hedgerows</td>
</tr>
<tr>
<td>Common shrew</td>
<td>Approximately 42–69/ha (deciduous woodland and grassland in summer)</td>
<td>0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Widespread and common</td>
<td>Thick grass, hedgerows, scrub and deciduous woodland</td>
</tr>
<tr>
<td>Stoat</td>
<td>Not known for the UK. But in Sweden 3–10/100 ha (rough pasture).</td>
<td>0.266&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Widespread</td>
<td>Most habitats with cover (e.g. farmland, hedgerows and dry stone walls</td>
</tr>
<tr>
<td>Polecat</td>
<td>Approximately 0.5–1/100 ha (Herefordshire farmland).</td>
<td>0.817&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Clumped. spreading south as far as Avon and west throughout Wales</td>
<td>Woodland, forest plantations. Frequently found around farm hedgerows and buildings (especially in winter</td>
</tr>
<tr>
<td>Fox</td>
<td>Approximately 0.3–5.1/km² (rural South-West England).</td>
<td>6.050&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Widespread and common</td>
<td>Almost ubiquitous. Frequent in mosaics of woodland, scrub and farmland</td>
</tr>
<tr>
<td>Red deer</td>
<td>Approximately 1–30/km² (open ground and woodland).</td>
<td>115.75&lt;sup&gt;d,p&lt;/sup&gt;</td>
<td>Patchy but locally abundant</td>
<td>Woodland, grassland and scrub, on upland moors in the Quantocks and Exmoor</td>
</tr>
<tr>
<td>Fallow deer</td>
<td>Highly variable and often associated with management, commonly 5–20/km²</td>
<td>57.75&lt;sup&gt;d,p&lt;/sup&gt;</td>
<td>Widespread but patchy</td>
<td>Favours mature deciduous or mixed woodland, close to open farm or parkland</td>
</tr>
<tr>
<td>Roe deer</td>
<td>Estimates vary widely from 6.9–101/km² (open ground and woodland), but typically between 15–25/km² (lowland England).</td>
<td>17.50&lt;sup&gt;p&lt;/sup&gt;</td>
<td>Widespread and common</td>
<td>Most frequent in woodlands and thickets, may feed in arable and open farmland</td>
</tr>
<tr>
<td>Muntjac deer</td>
<td>Approximately 15–30/km² (woodland).</td>
<td>13.75&lt;sup&gt;d,p&lt;/sup&gt;</td>
<td>Widespread but very clumped</td>
<td>Mainly in dense deciduous or mixed woodland and scrub</td>
</tr>
</tbody>
</table>

The density values used in the quantitative risk assessment are given in <sup>bold</sup>.<br/>

<sup>a</sup> Corbet and Harris (1991).  
<sup>b</sup> Kenward et al. (1998).  
<sup>c</sup> Macdonald et al. (1998).  
<sup>d</sup> Macdonald and Barrett (1993).  
<sup>e</sup> Harris et al. (1995).  
<sup>f</sup> Gurnell (1979).  
<sup>g</sup> Gurnell (1981).  
<sup>h</sup> Green (1979).  
<sup>i</sup> Flowerdew (1985).  
<sup>j</sup> Tapper (1979).  
<sup>k</sup> King (1989).  
<sup>l</sup> Central Science Laboratory (xxx).  
<sup>m</sup> Ward (2005).  
<sup>n</sup> Staines and Ratcliffe (1987).  
<sup)o</sup> Langbein (1997).  
<sup>p</sup> Whitehead (1993).  
<sup>q</sup> Langbein and Chapman (2003).  
<sup>r</sup> Putman (2003)  
<sup>s</sup> Ward, A.I., (personal communication).  
<sup>t</sup> ENACT 3 (1995).  
<sup>u</sup> Cooke et al. (1996).  
<sup>v</sup> Chapman and Harris (1996).
Table 9
A semi-quantitative risk assessment showing the potential risk of disease transmission to cattle, relative to the badger, from those mammal species in which infection was detected during the current study, compared to that of the badger

<table>
<thead>
<tr>
<th>Deer species</th>
<th>Prevalence (min, max)</th>
<th>Mean likelihood of excretion</th>
<th>Mean likelihood of contact with cattle</th>
<th>Biomass kg/km²</th>
<th>Final median risk score (inter-quartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey squirrel</td>
<td>0.44 (0.05, 1.60)</td>
<td>0.13</td>
<td>0.22</td>
<td>275</td>
<td>0.002 (0.001–0.006)</td>
</tr>
<tr>
<td>Yellow-necked mouse</td>
<td>2.78 (0.07, 14.53)</td>
<td>0.13</td>
<td>0.19</td>
<td>14</td>
<td>0.001 (0.000–0.003)</td>
</tr>
<tr>
<td>Wood mouse</td>
<td>0.60 (0.07, 2.15)</td>
<td>0.13</td>
<td>0.23</td>
<td>40</td>
<td>0.001 (0.000–0.001)</td>
</tr>
<tr>
<td>Field vole</td>
<td>1.49 (0.04, 8.04)</td>
<td>0.13</td>
<td>0.23</td>
<td>680</td>
<td>0.034 (0.011–0.092)</td>
</tr>
<tr>
<td>Common shrew</td>
<td>2.44 (0.06, 12.86)</td>
<td>0.13</td>
<td>0.19</td>
<td>45</td>
<td>0.003 (0.000–0.009)</td>
</tr>
<tr>
<td>Stoat</td>
<td>3.85 (0.80, 10.83)</td>
<td>0.17</td>
<td>0.13</td>
<td>2</td>
<td>0.000 (0.000–0.000)</td>
</tr>
<tr>
<td>Polecot</td>
<td>4.17 (0.11, 21.12)</td>
<td>0.17</td>
<td>0.23</td>
<td>1158</td>
<td>0.000 (0.000–0.000)</td>
</tr>
<tr>
<td>Fox</td>
<td>3.17 (2.04, 4.69)</td>
<td>0.27</td>
<td>0.42</td>
<td>12</td>
<td>0.004 (0.000–0.009)</td>
</tr>
<tr>
<td>Red deer</td>
<td>1.02 (0.12, 3.64)</td>
<td>0.92</td>
<td>0.6</td>
<td>1158</td>
<td>0.834 (0.266–2.135)</td>
</tr>
<tr>
<td>Fallow deer</td>
<td>4.37 (2.76, 6.53)</td>
<td>0.96</td>
<td>0.71</td>
<td>722</td>
<td>1.624 (0.808–3.123)</td>
</tr>
<tr>
<td>Roe deer</td>
<td>1.02 (0.47, 1.92)</td>
<td>1.00</td>
<td>0.63</td>
<td>462</td>
<td>0.262 (0.122–0.494)</td>
</tr>
<tr>
<td>Muntjac</td>
<td>5.17 (1.08, 14.38)</td>
<td>0.67</td>
<td>0.42</td>
<td>275</td>
<td>0.329 (0.119–0.812)</td>
</tr>
<tr>
<td>Badger</td>
<td>10.94 (9.76, 12.21)</td>
<td>1.00</td>
<td>1.00</td>
<td>54</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Prevalence was derived from confirmed positive cases in Table 1. Badger prevalence and associated confidence interval was taken from RTA data collected from 1994 to 1999 (Clifton-Hadley, R., unpublished data. The likelihood of excretion, and contact with cattle, are the mean values of the combined risks. Biomass is the product of the relevant density and bodyweight calculated from Table 8. The final risk value is the median, (and inter-quartile range) based on 10,000 simulations.

Table 10
A sensitivity analysis of the risks, relative to the badger, of *M. bovis* transmission to cattle from deer species identified as hosts in the present study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Deer species</th>
<th>Prevalence</th>
<th>Excretion</th>
<th>Contact with cattle</th>
<th>Density</th>
<th>Weight</th>
<th>Badger</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red</td>
<td>Fallow</td>
<td>Roe</td>
<td>Muntjac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence</td>
<td>13.0</td>
<td>2.5</td>
<td>7.0</td>
<td>8.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excretion</td>
<td>17.8</td>
<td>39.9</td>
<td>27.9</td>
<td>21.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact with cattle</td>
<td>30.7</td>
<td>15.3</td>
<td>33.3</td>
<td>53.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>25.3</td>
<td>14.8</td>
<td>2.2</td>
<td>1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Badger</td>
<td>12.4</td>
<td>26.7</td>
<td>28.8</td>
<td>14.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as the percentage contribution to the variance for each parameter for each species. The last parameter (Badger) refers to the variance in all of the parameters (prevalence, density and weight) for the badger.

of infection in foxes (1.2% compared to 3.2%) and a substantially higher prevalence in fallow deer (18.5% compared to 4.4%). However, direct comparisons are problematic as the MAFF investigations were unsystematic, included submissions of suspect cases, some deer came from farmed and enclosed populations and post mortem examination methods varied (Delahay et al., 2002). In contrast, in the present study every effort was made to request that carcasses of suspect cases were either not submitted or were clearly identified as such so that they could be omitted from prevalence calculations. Further sources of potential bias were reduced by omitting deer from farmed or enclosed populations from the analyses, and implementing a rigorous and systematic procedure for post mortem examinations (see Barlow et al., unpublished data). This maximised the likelihood that the sample was representative of the free-living populations of all the species collected. However, sources of collection could not be rigorously controlled as they relied heavily on voluntary suppliers. For example, the majority of deer carcasses were derived from animals culled in managed forests and estates, and consequently the composition of samples submitted probably reflected age and/or sex-specific culling related to the prevailing management regime. Nevertheless, collection of deer from throughout the South-West region over a three year period should have reduced the influence of such local biases.

In the present study, positive culture cases were only confirmed as *M. bovis* if a spoligotype was obtained from the isolate. For a substantial number of culture positive cases (*n* = 27; Table 2) however, no spoligotype could be identified and the reasons for this are unclear. One possibility is that in some cases mycobacteria other than *M. bovis* were isolated. For example, *M. avium* is known to infect deer (Mathews et al., 1981) and cannot be identified by spoligotyping. The status of these ‘unconfirmed cases’ is currently undergoing further investigation using a variety of molecular techniques.

Prevalence estimates for deer may have been underestimated because the likelihood of detecting lesions, and confirming *M. bovis* infection by culture and spoligotyping was significantly lower for samples that consisted of incomplete carcasses. This suggests that any future studies of *M. bovis* infection in deer, should involve the examination and culture of tissues from complete carcasses. However, as the majority of deer were shot as part of routine population management practices, with the intention to provide the meat to game dealers, we were more likely to receive complete carcasses of animals that were in poor health. Hence, this may have contributed to the observed differences in prevalence, but as we have no reason to believe that incomplete carcasses came from animals that were selectively shot, this should not invalidate pooling the samples to calculate prevalence.

In the present study, small sample sizes for some species resulted in prevalence estimates with wide confidence limits. Consequently, although the results indicate evidence
of infection in the polecat, common shrew, yellow-necked mouse and muntjac, the extent of infection in these species cannot be reliably estimated (see Table 1). Nevertheless, these estimates do provide some information on the likely minimum level of infection. Hence, for muntjac we can be 95% confident that the prevalence of infection in the population is between 1% and 14%. In contrast small sample sizes for species in which no positive cases were observed (e.g. house mouse (Mus domesticus), sika deer), provide very unreliable evidence for the absence of infection. In the case of sika deer M. bovis infection has already been confirmed in previous studies (Delahay et al., 2002). For some species the small sample sizes obtained were a reflection of their restricted range and low relative abundance, for example the otter (Lutra lutra), water shrew (Neomys fodiens), water vole (Arvicola terrestris), feral goats (Capra hircus) and feral sheep (Ovis aries). Hence, even if infection were identified in these species they would be unlikely to pose a significant risk to cattle.

Only a small number of feral wild boar (n = 7) were collected during the present study, and although numbers are currently low and their range highly restricted, it is thought that the population will continue to grow (Goulding et al., 2003). As M. bovis has been identified in wild boar in Italy (Serraino et al., 1999; Bollo et al., 2000) and Spain (Gortazar et al., 2003) and molecular epidemiological data suggests the transfer of infection between boar, deer and cattle (Serraino et al., 1999), it may be necessary to review the potential role of wild boar in the UK as the population grows.

No evidence of infection was detected in hedgehogs, rabbits (Oryctolagus cuniculus), bank voles (Clethrionomys glareolus) and brown rats, despite relatively large sample sizes. The failure to detect infection in brown rats contrasts with previous investigations by MAFF which identified five positive individuals in a sample of 412 (see Delahay et al., 2002) although in the latter case a high proportion of animals were taken from farmyards.

For some species in which infection was confirmed, relatively large sample sizes were obtained, resulting in prevalence estimates with relatively narrow confidence limits (e.g. wood mouse, grey squirrel, fox, roe and fallow deer; see Table 1). The highest prevalence estimates were obtained for fallow deer (4.37%) and muntjac (5.17%), although in the latter wide confidence limits resulted from the small sample size. As would be expected for a chronic disease such as bovine tuberculosis, the prevalence of infection was greater in adult fallow and roe deer, although no such difference could be detected for foxes. However, prevalence in foxes was significantly higher amongst females. It is not clear whether this is because of a difference in susceptibility or enhanced disease-induced mortality amongst males. The latter is unlikely as severe pathology was not observed in any foxes collected in either the present or previous studies (Delahay et al., 2002).

Positive cases were found in wild mammals throughout the South-West region although they tended to originate from where most carcasses were collected (i.e. Gloucestershire and Herefordshire). Prevalence of infection did not differ between triplet areas of the RBCT for foxes, red and roe deer, although for some areas sample sizes were small. However, there were significant differences in prevalence amongst fallow deer. Infection was detected in fallow deer collected in Gloucestershire (23 cases in 325) and Herefordshire (1 case in 16) but was absent from those from Somerset (n = 53) and Devon (n = 74). Investigation of the prevalence of infection in smaller areas showed substantial variation (see Table 5). For example a prevalence of 2.7% was found for roe deer in an area of approximately 25 km² in Gloucestershire but was absent from those collected in an area over twice as large in the Mendips, Somerset. The spatial clustering of M. bovis infection has been observed before in wildlife populations and can be problematic for the estimation and interpretation of prevalence data (Delahay et al., 2000). The pathological effects of M. bovis infection vary widely between host species (Thorns et al., 1982; Delahay et al., 2002). Consequently, in the present study a variety of tissues and organs and all potentially suspicious lesions and/or abnormalities were collected for microbiological culture. As all suspicious tissues were cultured separately, this maximised the sensitivity of the methods for detecting M. bovis. However, in 64.6% of cases where suspect lesions were detected, M. bovis could not be isolated by subsequent culture. Although culture is unlikely to detect M. bovis in all cases, the broad definition of a suspect lesion adopted in the present study was likely to result in the collection of some non-tuberculous lesions. For example, Yersinia pseudotuberculosis infection, which has been observed in wild deer (Chapman et al., 1979), was observed several times during the present survey (Barlow, A., personal observation) and can produce lesions that closely resemble those of M. bovis. Also, a high proportion of confirmed positive cases had no visible lesions. Together these factors suggest that the presence of lesions alone is not a good predictor of M. bovis infection in wild mammals. Possible exceptions to this were lesioned red and fallow deer, which accounted for the overwhelming majority of confirmed positive cases.

In the majority of infected foxes and stoats collected in the present study, gross pathology was not detected although in a single case from each species macroscopic lesions were observed in the mesenteric lymph nodes. Although details of the pathogenesis of infection in these species are unknown, lesions in the mesenteric lymph nodes would be consistent with infection by ingestion (of infectious carrion perhaps). In addition, if ingested bacilli were to produce non-macroscopic lesions in the wall of the small intestine (which would have remained undetected in the present study), these could be a source of excreted bacilli in faeces. Evidence from New Zealand suggests that feral ferrets become infected via the alimentary tract, potentially giving rise to excretion of bacilli via the oral, faecal and urinary routes (Lugton et al., 1997). Alternatively infection could be transmitted between badgers and foxes as a consequence
of sharing dens (Macdonald, 1987; Neal and Cheeseman, 1996) or feeding areas (Macdonald et al., 2004).

The most extensive pathology observed in the present study was associated with *M. bovis* infection in fallow, roe and red deer. Gross pathology was only observed in one muntjac, which had confirmed lesions in the lungs and the pre-scapular lymph nodes, and unconfirmed lesions in the bronchial and mediastinal lymph nodes. In fallow, red and roe deer the principal sites of infection were also in the lungs and the associated lymph nodes, consistent with infection by inhalation (Beatson, 1985; Williams, 1987; Fleetwood et al., 1988; Stuart et al., 1988) although the tonsils have also been implicated as a route of infection in deer (Mackintosh et al., 1993). The presence of extensive lesions in a high proportion of positive fallow, red and roe deer (see Table 7) is consistent with the potential for onward transmission. Gross lesions were generally more florid in deer although their potential for bacterial excretion is unclear. Histopathology was not carried out but tissues were archived for future examination to provide further information on the pathogenesis of infection and the potential for excretion. It is not yet known if the rate of intra-specific transmission is sufficient to maintain the disease in UK deer populations in the absence of an external source of infection. Nevertheless, maintenance of infection is likely to be enhanced in the more gregarious species such as fallow and red deer.

No confirmed gross lesions were found in small mammals, grey squirrel or polecat despite the presence of confirmed positive cases. Obviously there are practical problems in detecting gross lesions in smaller species. Hence, there is inadequate information to assess the likely route of infection in these species. However, one possible route of infection for small mammals and grey squirrels could be badger latrines as these species have been observed foraging on undigested cereal in badger faeces (Delahay, R.J., Cheeseman, C.L., personal observation). In North America, raccoons (*Procyon lotor*) are implicated in the transmission of a nematode parasite to the granivorous mammals that visit their latrines to forage on undigested seeds (Page et al., 1999).

Determining whether maintenance of infection and onward transmission occurs in any particular species would require knowledge of levels of infection in the absence of transmission from other species. However, this would be logistically difficult in the UK given the relatively broad range of wild mammal hosts. The isolation of common *M. bovis* spoligotypes from a variety of wild mammal species in the present study is consistent with inter-species transmission, although it is not possible to speculate on the likely direction. The frequency of occurrence of different spoligotypes in wild mammals examined during the present study was similar to that found in cattle and badgers, which is consistent with transfer of infection amongst these species. Type 17 was the most commonly observed spoligotype in cattle, badgers and wild mammals in Gloucestershire. Type 10 isolates however, were largely confined to a localised cluster of fallow deer in part of Gloucestershire. Hence, there is some evidence to suggest the transfer of infection between species, and the existence of localised clusters of infection. Investigation of the spatial associations between *M. bovis* positive cattle, badgers and other wild mammals is the subject of further analyses.

In order to assess the likely risks to cattle posed by *M. bovis* infection in wild mammals, information on the prevalence of infection, pathology, and the ecology and density of the wildlife hosts was considered. An initial semi-quantitative risk assessment demonstrated that the risk from deer species, relative to the badger, was potentially substantial, particularly for fallow and red deer. However, given wide variation in deer densities (Harris et al., 1995) and the patchy distribution of fallow and red deer in particular in South-West England (Ward, 2005), this risk is by definition likely to be localised. Hence, the results suggest that, at least locally, these deer species may constitute a risk to cattle similar to that from badgers, although for both species the uncertainty of the risk is very high. Their potential importance and high levels of uncertainty associated with current estimates suggest that further work (e.g. histopathology) is required to better determine the level of bacterial excretion by deer, and that an improved assessment is performed on their relative ability for either direct or indirect contact with cattle.

Although sample sizes for some species were inadequate to rule out their potential involvement, it seems unlikely that most species in which infection was not detected in the present study, pose a significant risk to cattle. In most of the species in which infection was confirmed, particularly small mammals, the likely risks to cattle also appear relatively low, although further investigation of the pathology of infection in small mammals should be carried out before there is sufficient evidence to rule them out. The roles of polecats, stoats and foxes are also unclear, although the relative scarcity of the former and infrequency of pathology in all three suggests that they are unlikely to represent a high risk to cattle. The present study did however identify pathology and levels of infection in fallow and red deer, and in some locations in roe deer also, that indicate a potential risk of disease transmission to cattle, which was relatively high in comparison to the other species surveyed. The limited number of sika deer means also that they cannot be ruled out as a means of transmission to cattle.

In addition, prevalence values for deer given in the present study may be underestimates owing to the lower probability of detecting infection in incomplete carcasses. Nevertheless, none of the estimates of *M. bovis* prevalence for wild mammals in the present study approach those observed in badgers. For example, in the vicinity of the Woodchester Park study area the prevalence of infection detected in RTA badgers at post mortem during the period 2000–2003 inclusive was 20.5% (Delahay, R.J., Cheeseman, C.L., unpublished data). In addition, badgers are known to excrete potentially large numbers of bacilli (Gallagher et al., 1976; MAFF, 1976–97) and to forage on pasture
(Kruuk et al., 1979) and in buildings used by cattle (Garnett et al., 2002).

Nevertheless, the risk assessment suggested a high potential risk of transmission of infection from deer to cattle, relative to badgers. In the past, deer have been implicated in the transmission of bovine tuberculosis to cattle in the UK (Anon, 1984; Gunning, 1985) and in particular localities, especially where population density is high, they could pose a significant risk. In the light of the results presented here, the paucity of data on interactions between deer and cattle and their rapidly expanding numbers and distribution in southern England, it seems prudent to consider deer as a potential, although probably localised source of infection for cattle.

Acknowledgements

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Appendix 1. Sources of data for Table 8


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Central Science Laboratory. Unpublished survey data.


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