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MYCOBACTERIUM BOVIS IN WILDLIFE IN FRANCE

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ABSTRACT: In early 2001, tuberculosis-like lesions were detected in three hunter-killed red deer (*Cervus elaphus*) in the Brotonne Forest (Normandy, France), and *Mycobacterium bovis* was isolated. In subsequent hunting seasons, two surveys were conducted in the area. In the first survey (2001–02 hunting season), nine (13%) of 72 red deer sampled were positive for *M. bovis*. In the 2005–06 hunting season, the prevalence of *M. bovis* infection increased to 24% (χ^2=3.85, df=1, P=0.05; 33 positive among 138 sampled). The prevalence remained stable in juveniles, but it increased significantly in adults: from 13% in 2001–02 to 32% in 2005–06 (χ^2=5.13, df=1, P=0.02). Wild boar (*Sus scrofa*) were heavily infected in both surveys. One roe deer (*Capreolus capreolus*) and one red fox (*Vulpes vulpes*) also tested positive in the second survey. *Mycobacterium bovis* was not isolated from Eurasian badgers (*Meles meles*). Spoligotyping and mycobacterial interspersed repetitive unit-variable number tandem repeat analysis demonstrated that all *M. bovis* strains isolated from wildlife were of the same genotype. Thus, the wildlife outbreak involved only a single strain, and this strain was the same as that circulating in nearby cattle herds since 1995. Sensitivity, specificity, and predictive values of the presence of macroscopic lesions as a diagnostic criterion were evaluated from the data obtained from red deer. Necropsy seems to be satisfactory as a routine tool to monitor the disease in wild red deer populations in which bovine tuberculosis has become established.

Key words: *Cervus elaphus*, diagnostic tests, France, molecular typing, *Mycobacterium bovis*, red deer, *Sus scrofa*, wild boar.

INTRODUCTION

There has been a significant decrease in the incidence of bovine tuberculosis in domestic livestock in countries where test and slaughter control measures have been implemented. However, eradication of bovine tuberculosis using this approach is not always possible in areas where wildlife reservoirs exist. Bovine tuberculosis has been reported in various wildlife species, and wildlife reservoirs for *Mycobacterium bovis* have been documented. The existence of such reservoirs is dependent upon unique ecologic factors and species susceptibility (Morris et al., 1994). The criteria to be considered when assessing whether a given wildlife species is a reservoir host includes 1) a self-sustaining infection within that species population, 2) a clear spatial and temporal association between infection (with specific strains confirmed by molecular typing) in that host and domestic livestock, and 3) a reduction in the incidence of infection in livestock associated with an elimination or reduction of the wildlife reservoir population (Morris et al., 1995). In Europe, the best-documented reservoir for *M. bovis* is the Eurasian badger (*Meles meles*) in the UK (Gallagher and Clifton-Hadley, 2000; Simpson, 2002). Infections with *M. bovis* have been reported from red deer (*Cervus elaphus*) in Spain, where that species is considered to be a reservoir host (Parra et al., 2005; Vicente et al., 2006). Other wildlife reservoirs include brushtail possums (*Trichosorus vulpecula*) in New Zealand (Buddle et al., 1994; Coleman and Cooke, 2001) and free-ranging white-tailed deer (*Odocoileus virginianus*) in Michigan, USA (O'Brien et al., 2002; Palmer et al., 2004).

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France was officially declared bovine tuberculosis free in 2000 by the European Commission, and at that time, there was no indication of a wildlife reservoir. In early 2001, macroscopic tuberculosis-like lesions in lung and liver were observed in three red deer that were shot by hunters in the Brotonne Forest, Normandy. *Mycobacterium bovis* infection was confirmed by culture from affected organs. An epidemiologic survey in the following hunting season (2001–02) confirmed the presence of bovine tuberculosis in both red deer and wild boar (*Sus scrofa*). Control measures, such as the reduction of red deer population levels, a ban on supplemental feeding, and the destruction of animal viscera from harvested animals, were implemented in late 2002. Fencing to reduce contact between wild animals and cattle in the vicinity of the forest was also extended. In the following years, passive surveillance data on hunter-killed or dead animals with tuberculosis-like lesions confirmed the continued presence of *M. bovis* circulation in these species. During the 2005–06 hunting season, a second survey was conducted to evaluate the progression of the disease in the affected species and the effect of control measures; this work included an assessment of spillover of the infection into other species. There was an apparent increase of the incidence in domestic animals between 1996 and 2003, and several outbreaks of unknown origin were reported affecting cattle farms around the Brotonne Forest.

The presence and apparent establishment of *M. bovis* in red deer and wild boar in France raise issues related to its control and to technical aspects of *M. bovis* surveillance. Although *M. bovis* culture is regarded as the "gold standard" for diagnosis, culture-based diagnostics may be too slow, time-consuming, and expensive to use for monitoring infection in a wildlife population. Alternative methods for the identification of infected animals would be very useful, but the diagnostic performance (sensitivity, specificity, positive, and negative predictive values) of any such methods would need to be good. Necropsy examination (occurrence of tuberculosis-like gross lesions) is one such approach that has been used to evaluate the prevalence of tuberculosis in wild boar and wild red deer in Spain (Parra et al., 2005; Vicente et al., 2006).

In this study, we present results for the 2001–02 and 2005–06 surveys for *M. bovis* in wildlife populations in the Brotonne and Mauny forests, Normandy, France. Molecular typing of isolates was used to link wildlife and domestic livestock outbreaks. Data from these surveys were also used for the first evaluation of the sensitivity, specificity, and positive and negative predictive values of necropsy examination for the detection of bovine tuberculosis in wild red deer. We show that this approach can be used as a diagnostic criterion, instead of bacterial culture results, for populations where *M. bovis* is known to be present.

MATERIALS AND METHODS

Study area and sampling

The study area includes the Brotonne Forest (81 km^2) and the nearby Mauny Forest (10 km^2), both in Normandy (49°21′–49°27′N, 0°51′–0°55′E). A land strip that connects the two forests allows the movement of wild animals between them. Both forests are bounded by the Seine River except in the south where their borders are a highway. Therefore, we considered the movement of wild animals between these two and other forests of the region to be negligible. The Brotonne and Mauny forests are devoted to "recreational" deer and boar hunting. The habitat is characterized by conifers and broad-leaved trees. Domestic livestock, mainly cattle, are extensively reared on the boundaries of both forests.

In the 2001–02 hunting season, wild ungulates were tested; sample size was sufficient to detect a prevalence of at least 3% in each species with 95% confidence. The species included were red deer, roe deer (*Capreolus capreolus*), and wild boar.

For red and roe deer, population size was estimated by spotlight counts combined with the harvest data from the previous hunting

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season. The wild boar population was estimated from harvest data during the previous year. A target sample size for 90 animals of each species was established based on these approximate population sizes, with the aim of obtaining a balanced distribution according to sex and age.

For the 2005–06 hunting season, target sample sizes were 150 red deer, 150 wild boars, and 50 roe deer. Red foxes (*Vulpes vulpes*) and badgers were also sampled and tested; these also were hunter-killed animals.

Hunters were asked to cooperate by presenting harvested animals for necropsy, and in both surveys, almost all red deer carcasses were made available. For wild boars, carcasses were provided by hunters after evisceration; this may have reduced the probability of detecting gross lesions. Necropsy examination of all animals of all species was performed by a veterinarian, and samples were collected from tuberculosis-like lesions and systematically from the respiratory tract and retropharyngeal lymph nodes. In 2005–06, mesenteric lymph nodes were also collected from each carcass. In addition to necropsy data, species, date of collection, location, sex, and age were recorded for each animal.

Prevalence of *M. bovis* infection and of tuberculosis-like lesions

The prevalence of tuberculosis-like lesions (based on necropsy and subsequent examination of collected tissues) and the prevalence of *M. bovis* infection (based on *M. bovis* isolation) in red deer were calculated for each hunting season. We considered that red deer were sampled randomly but that wild boars were not; hunters probably submitted a larger proportion of animals presenting macroscopic lesions at evisceration than of animals without visible lesions. Consequently, the prevalence computed from this data set is likely to be overestimated.

Bivariate analyses were used to test for associations between infection status and sex and age class (juveniles, $\leq$2 yr; adults, $>$2 yr) only for the red deer data. Wild boar samples were considered not to be representative. Animals for which age was not available were excluded from the analysis. The statistical software package SAS version 8 (SAS Institute Inc., Cary, North Carolina, USA) was used for prevalence and contingency table analyses.

Sensitivity, specificity, and predictive values of necropsy used as a diagnostic tool

Sensitivity, specificity, positive predictive value, and negative predictive value are standard indicators of the performance of a diagnostic test (Dohoo et al., 2003), and they were calculated for tuberculosis-like lesions using culture as the gold standard. Separate analyses were done for sex, age class, and survey year but only for red deer. A statistical software package (SAS) was used to estimate sensitivity, specificity, and predictive values.

Culture, identification methods, and molecular typing

The respiratory tract was carefully dissected in the laboratory, and the lymph nodes were recovered. Each type of lymph node (retropharyngeal, trachea, lung, and mesenteric nodes) and tissue with gross lesions were individually packed, labeled, and cultured. Lymph nodes were sectioned and examined for gross lesions, which were recorded if present. Tissue was sliced with a scalpel blade and homogenized by stomaching in the presence of 4% sulfuric acid as decontaminating solution. The samples were neutralized with 6% sodium hydroxide and used to inoculate Lowenstein-Jensen and Coletsos egg-based solid media at 30 C, 37 C, and 42 C. The tubes were observed a week after inoculation and subsequently every month for 3 mo. All colonies were noted, and if present, they were stained by the Ziehl-Neelsen method. If acid-fast bacteria were detected, the *Mycobacterium* sp. was identified by classical biochemical and culture methods (Kent and Kubica, 1985); *M. tuberculosis* and *M. avium* complexes were confirmed by DNA amplification as described by Hénault et al. (2006).

Molecular typing techniques were used for all *M. bovis* strains isolated from wildlife and for strains that were isolated from tuberculosis outbreaks among cattle in the region surrounding the Brotonne and Mauny forests. Additional strains from the National Reference Laboratory (associated with other outbreaks in cattle in other parts of Normandy), with the same spoligotype as the wildlife strain, were also included in the analyses.

The spoligotyping method was done as described previously (Kamerbeek et al., 1997). Spoligotyping is the major technique used for *M. bovis* typing based on the characterization of the direct repeat (DR) region. The DR region is a monolocus area that is specific to the *M. tuberculosis* complex. Polymerase chain reaction (PCR) amplification of the DR locus reveals the presence/absence of each of 43 variable areas of the DR locus. Heat-treated *M. bovis* cell suspensions were used. Biotin-labeled PCR products were detected on a spoligotyping membrane (Isogen Bioscience BV, Maarssen, The Netherlands). Purified sterile water and *M. bovis* and

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M. tuberculosis controls included in the spoligomembrane kit were included in each batch of tests. The nomenclature used by www.Mbovis.org database was adopted.

Another and more discriminative molecular typing method is the mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR; Skuce et al., 2002). It is a multiallelic system combining the characterization of several independent regions, scattered along the mycobacterial genome and evolving independently of each other. For each of these loci, MIRU-VNTR analysis reveals the number of repeats of a basic unit. We used this technique for amplification of loci QUB 11a, 11b, 26, and 3336 and loci ETR-A, ETR-B, ETR-C, and ETR-D, which in our hands are the most polymorphic among *M. bovis* French cattle strains (data not shown).

For PCR, a final volume of 30 μl was used with previously reported primer sets (Skuce et al., 2002). Each PCR mix contained 3 μl of 10X PCR buffer (QIAGEN S.A., Courtaboeuf, France), 1.5 mM $MgCl_2$, each primer at 0.66 μM, each of the four deoxynucleoside triphosphates at 200 μM, 6 μl of 5X Q-solution (QIAGEN S.A.), and 1 U of Hot StarTaq DNA polymerase (QIAGEN S.A.). Amplification was performed in a PerkinElmer GeneAmp 9600 (PerkinElmer Life and Analytical Sciences Boston, Massachusetts, USA) using 2 μl of template DNA. After initial denaturation at 95 C for 15 min, the PCR mixes were subjected to 30 cycles of 94 C for 30 sec, 55 C for 60 sec, and 72 C for 90 sec. Positive and negative control reactions, in which PCR mixes were inoculated with 2 μl of boiled cells of *M. tuberculosis* strain H37Rv and sterile distilled water, respectively, were performed with each set of reactions. PCR products were separated electrophoretically on 1% NuSieve GTG (Flowgen FMC Bioproducts, Rockland, Maine, USA) and 1% typing-grade SeaKem agarose (Flowgen FMC Bioproducts). A 100-bp DNA ladder (Euromedex, Mundolsheim, France) was used as size markers. Allele naming table for each tandem repeat locus was calculated, and allele variations were named using the copy number of the repeat unit present (Frothingham and Meeker-O'Connell, 1998; Skuce et al., 2002).

RESULTS

Prevalence of tuberculous lesions and *M. bovis* in red deer

In the 2001–02 survey, 80 randomly selected red deer from 94 available hunter-killed deer were inspected for *M. bovis*. Eight animals, for which the age was not available, were excluded from the analysis. The prevalence of gross tuberculous lesions was 18% (95% confidence interval [CI]: 8.6–26.7%). In the 2005–06 survey, gross lesions were found in 34 of 138 red deer sampled for which age was available (seven animals of unknown age were excluded); 149 animals were presented by hunters, and the prevalence of gross tuberculous lesions was 25% (95% CI: 17.5–31.8%). Prevalence estimates for the two surveys were not significantly different (Table 1).

In the 2001–02 survey, 13% (95% CI: 4.9–20.1%) of the sampled animals were cultured positive for *M. bovis*. The prevalence of the infection was higher (24%; 95% CI: 16.8–31.0%, $\chi^2=3.85$, df=1, $P=0.05$) in the 2005–06 sample. This increase in prevalence was most pronounced in adults; from 13% in 2001–02 to 32% in 2005–06 ($\chi^2=5.13$, df=1, $P=0.02$), whereas the prevalence in juveniles remained stable (11% and 14%, respectively). Prevalence estimates exceeded 10% for all age classes and in both sexes, except for juvenile females during 2001–02; this cohort was not represented in the sample from that survey (Table 1).

In 2005–06, there was a significant association between age and *M. bovis* infection (Table 1), with the prevalence in adults being nearly twice as high (32%) as that in juveniles (14%; $\chi^2=6.07$, df=1, $P=0.01$). In 2001–02, the prevalence in juveniles was similar to that in adults (11% and 13%, respectively). No association was found between gender and *M. bovis* infection in either survey.

Prevalence tuberculous lesions and *M. bovis* in wild boar and other species

For wild boar, 29% and 42% of tested animals were culture positive for *M. bovis* during 2001–02 and 2005–06, respectively (Table 2). Gross lesions suggestive of tuberculosis also were present in 29% of the animals examined in 2001–02 and in 42% of

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TABLE 1. Prevalence of gross lesions and prevalence of infection among red deer by survey year, age class, and sex.

Hunting season	Age class (yr)	Females			Males			Total		
		No. with gross lesions (%)	No. culture positive (%)	*n*	No. with gross lesions (%)	No. culture positive (%)	*n*	No. with gross lesions (%)	No. culture positive (%)	*n*
2001–02[a]	Juveniles (≤2)	1 (8)	0 (0)	13	3 (23)	3 (23)	13	4 (15)	3 (11)	27
	Adults (>2)	4 (17)	4 (16)	25	4 (27)	2 (18)	17	8 (19)	6 (13)	45
	Total	5 (14)	4 (11)	38	7 (25)	5 (17)	30	12 (18)	9 (13)	72
2005–06	Juveniles (≤2)	6 (22)	4 (15)	27	2 (6)	4 (13)	32	8 (14)	8 (14)	59
	Adults (>2)	11 (24)	11 (24)	45	15 (44)	14 (41)	34	26 (33)	25 (32)	79
	Total	17 (24)	15 (21)	72	17 (26)	18 (27)	66	34 (25)	33 (24)	138

[a] Sex not known for four animals; no necropsy data for two males and two females.

the animals in 2005–06. Gross lesions and infections were detected in both young and adult animals and in both males and females.

None of 38 roe deer examined in 2001–02 presented macroscopic lesions or were positive for *M. bovis* by culture (Table 2). Of 53 roe deer examined in the 2005–06 survey, a single 2-yr-old female roe deer with no macroscopic lesions tested *M. bovis* positive by culture. A single red fox was culture positive among the 49 tested. All badgers were culture negative (Table 2).

Sensitivity, specificity, and predictive values of necropsy used as a diagnostic tool

Complete gross pathology information and *M. bovis* culture results by age class were obtained for 68 red deer in 2001–02 (Table 3) and for 138 animals in 2005–06 (Table 4). Of 42 culture-positive animals, 36 presented gross lesions, corresponding to a sensitivity of 86%. However, sensitivity varied by survey within the adults age class; sensitivity was 50% in 2001–02 and 96% in 2005–06 (Fisher's test, $P=0.02$). This difference was not observed for juveniles where sensitivity was roughly the same for the two surveys (the number of animals, however, was very low). Variation was also observed with the positive predictive values; the overall proportion of culture-positive animals among animals presenting gross lesions was 78%, but the positive predictive value for adults was 37.5% for the first survey and 92.3% for the second (Fisher's test, $P=0.004$). The positive predictive value for juveniles was similar in the two surveys (75%).

Differences were not observed either for the specificity, or for the negative predictive value. The overall proportion of animals without lesions among culture-negative animals (specificity) was 94%. Similarly, the proportion of culture-negative animals among the animals without lesions (negative predictive value) was 96%.

M. bovis molecular typing and other mycobacteria

All *M. bovis* strains isolated from wildlife showed the same spoligotype

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TABLE 2. Numbers of animals hunted, sampled and that were *M. bovis* culture positive.

Hunting season survey	No. of animals	Wild boar	Roe deer	Badger	Red fox
2001–02	Sampled	85	38	0	0
	Culture positive	25	0	0	0
2005–06	Sampled	155	53	55	49
	Culture positive	65	1	0	1

pattern: SB0134. Strains isolated from cattle outbreaks near the Brotonne and Mauny forests and the wildlife strains all gave identical MIRU-VNTR profiles, whereas strains from other parts of Normandy had clearly different profiles, with a minimum difference between profiles of two loci (Table 5).

Other mycobacteria were isolated in animals that were negative to *M. bovis* in both surveys; these included 19 *M. avium* isolates (eight from red deer, seven from wild boars, and four from roe deer) and 60 isolates of unidentified nontuberculous mycobacteria (NTM; 19 from red deer, 33 from wild boars, and eight from roe deer). Mixed mycobacterial infections were identified in four cases: unidentified NTM and *M. bovis* in two red deer, unidentified NTM plus *M. bovis* in a wild boar and *M. avium* and *M. bovis* in the only positive red fox.

DISCUSSION

In this study, as is common when dealing with free-ranging populations, the sampling bias is unknown. Nevertheless, our findings provide evidence that the prevalence of *M. bovis* infection is high in the red deer population in the area studied. Unfortunately, wild boars were not randomly selected, and the prevalence of macroscopic lesions and infection could not be computed in the same way as for red deer. However, the large percentage of infected animals in the samples and the observation of macroscopic lesions in even young animals all indicate that *M. bovis* is circulating in wild boars. Only one roe deer and one red fox in the second survey were found to be infected; the infection is thus present in these species but probably at a very low level.

The overall prevalence of infection in red deer increased from 13% in 2001–02 to 24% in 2005–06. Analysis of the prevalence by age class suggests that the epidemiologic situation progressed from an early stage in 2001–02 (infection prevalence estimates by class age were similar) to a more advanced stage in 2005–06. Indeed, as time elapses there is an

TABLE 3. Sensitivity, specificity, and predictive values of the presence of gross lesions using culture as the gold standard in the 2001–02 survey in red deer by age class.

		Culture		
	Gross lesions	Positive	Negative	Total
Juveniles	Present	3	1	4 (75%)[a]
	Absent	0	22	22 (100%)[b]
	Total	3 (100%)[c]	23 (96%)[d]	26
Adults	Present	3	5	8 (38%)[a]
	Absent	3	31	34 (91%)[b]
	Total	6 (50%)[c]	36 (91%)[d]	42

[a] Positive predictive value.

[b] Negative predictive value.

[c] Sensitivity.

[d] Specificity.

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TABLE 4. Sensitivity, specificity, and predictive values of the presence of gross lesions using culture as the gold standard in the 2005–06 survey in red deer by age class.

		Culture		
	Gross lesions	Positive	Negative	Total
Juveniles	Present	6	2	8 (75%)[a]
	Absent	2	49	51 (96%)[b]
	Total	8 (75%)[c]	51 (96%)[d]	59
Adults	Present	24	2	26 (92%)[a]
	Absent	1	52	53 (98%)[b]
	Total	25 (96%)[c]	54 (96%)[d]	79

[a] Positive predictive value.

[b] Negative predictive value.

[c] Sensitivity.

[d] Specificity.

increasing probability of acquisition of infection because bovine tuberculosis is a chronic disease that is not highly lethal. Consistent with these observations, the proportion of animals with gross lesions among infected adults increased significantly (doubled) between the two surveys. An increase of the prevalence in juveniles would be expected in view of this increase in adults; however, it remained stable. If the prevalence in juveniles is taken as an indicator of the incidence of recent infections in the population, these findings suggest that the control measures implemented had some impact, as the incidence did not increase. However, the trends of prevalence will have to be followed in future surveys if this possibility is to be confirmed.

Bacterial culture significantly increases both overall effort, cost, and time associated with monitoring *M. bovis* in known infected populations. In our experience, acid-fast staining, although inexpensive, is a poor method for mycobacterial detection, and PCR analysis, which has the advantage of being a rapid diagnostic tool, is not suitable for testing for mycobacteria in gross lesions; Hénault et al. (2006) reported that a PCR method for detecting tuberculosis in wildlife was 18.5% less sensitive than culture. Therefore, diagnostic methods based entirely or partly upon necropsy results that are easier to collect

TABLE 5. MIRU-VNTR allele profiles (in order loci ETR A, ETR B, ETR C, ETR D, QUB 3336, QUB 11a, QUB 11b, and QUB 26) of *M. bovis* cattle strains (spoligotype SB0134) from Normandy, France. Wildlife strain profiles shown in bold.

Yr	No. of outbreaks	Epidemiologic link with wildlife outbreak	MIRU-VNTR profile
1979	1	No	3 3 3 4 9 9 4 6
1983	1	No	3 4 5 7 10 11 3 5
1995	11	Yes	**7 4 5 4 10 9 4 6**
	1	No	2 4 5 4 10 9 3 6
1996	1	Yes	**7 4 5 4 10 9 4 6**
	1	No	6 5 5 4 10 9 4 6
	1	No	4 5 5 4 10 9 4 6
1998	2	Yes	**7 4 5 4 10 9 4 6**
1999	1	Yes	**7 4 5 4 10 9 4 6**
2004	1	Yes	**7 4 5 4 10 9 4 6**
2006	2	Yes	**7 4 5 4 10 9 4 6**

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would be a valuable complement to laboratory analysis. Thus, it is important to evaluate the bias in the estimation of prevalence when necropsy results are used. Our data enabled us to estimate the epidemiologic performance of necropsy as a diagnostic tool in wild red deer using bacterial culture results being the gold standard. Reasons for discordance between culture and macroscopic lesions in red deer have been reported previously (Schmitt et al., 1997; Lugton et al., 1998; Mackintosh et al., 2004), and performance indicators for this tool have been evaluated in a previous study (Rohonczy et al., 1996) of farmed red deer. Results from the Rohonczy et al. (1996) study were similar to those obtained from overall data in our study, but this previous work did not consider age class or other epidemiologic situations. In our study, the results differed substantially according to the year of the survey and the animal's age. Sensitivity in adults was higher in the second survey (96%) than the first (50%), whereas it was higher for juveniles in the first (100%) than in the second (75%). In juveniles, the positive predictive value (proportion of animals with gross lesions that were culture positive) remained stable at a moderate level (75%), whereas in adults, it increased significantly and markedly from a very low level in 2001–02 to a satisfactory value in 2005–06. The specificity and negative predictive values were high for all age classes and in both surveys. Apparent prevalence, estimated by necropsy results, would have overestimated true prevalence (according to the gold standard) by 39% in juveniles and 44% in adults in the first survey, whereas in the second survey, the overestimation of the prevalence was 4% in adults and null in juveniles. This suggests that the epidemiologic situation should be taken into account when choosing the diagnostic tool. In particular, using necropsy as a diagnostic tool could lead to severely biased estimates in a population in which the epizootic is at an early stage; this bias seems to be much smaller for an epizootic at a more advanced stage. Hence, bacterial culture could be replaced by necropsy in wild red deer to reduce the costs, providing that its sensitivity and specificity are monitored using a sample of animals.

Molecular typing demonstrated that wildlife isolates and the isolates from cattle outbreaks around the Brotonne and Mauny forests are identical. Their spoligotype, SB0134, previously named GB35, is widespread among *M. bovis* strains from cattle outbreaks in France (Haddad et al., 2001). Because this typing method alone does not prove an infectious link between domestic and wild animals, MIRU-VNTR analysis was also performed. Cattle outbreaks strains from other parts of Normandy with the same SB0134 spoligotype clearly gave divergent MIRU-VNTR profiles; the Brotonne and Mauny strains from all host species gave identical patterns. Therefore, a single strain is associated with the wildlife outbreak, and it is the same as that circulating in nearby cattle herds since at least 1995. Wherever tuberculosis involves wildlife, the question of the identity of the reservoir species is raised. In our surveys, *M. bovis* was isolated from four species, and their epidemiologic status has yet to be established. Red deer and cattle often share the same pastures around the Brotonne Forest. It is likely that the infection was originally transmitted from cattle, the most abundant and natural host of *M. bovis*, to wild red deer, when the prevalence of infection was high in livestock of the region. The infection then spread within the red deer population. The high infection prevalence estimates for red deer, including juveniles, without evidence of a livestock source of transmission, suggest that the disease is self-maintaining in this population. Also, there have been livestock outbreaks of unknown origin near the Brotonne Forest, and they may well have been consequences of contact with infected red deer. Therefore, red deer may well be acting as a reservoir

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host, and further transmission from wildlife to cattle cannot be excluded. The role of wild boar is less clear. In other areas where potential reservoir hosts are present, like brushtail possums, feral buffaloes, or wild deer, it is assumed that feral pigs become infected by scavenging infected carcasses or offal of these species (Corner et al., 1981; Wakelin and Churchman, 1991; de Lisle, 1994); they are considered to be very susceptible to *M. bovis* (Nugent et al., 2002). In the Brotonne and Mauny forests, once the infection became established in red deer, it may have propagated into the wild boar population through scavenging of contaminated red deer offal left by hunters. If wild boar are spillover hosts, collection and destruction of hunted animal viscera, a policy implemented since 2002, should reduce *M. bovis* transmission from red deer to wild boar and lead to a decrease of infection in this species. Therefore, successive surveys with random samples of animals are needed to monitor the trend of the disease through time in wild boar and to elucidate its epidemiologic role. The very low prevalence of infection in both roe deer and red fox populations, suggests that these species are spillover hosts.

The *M. bovis* tuberculosis outbreak described here is currently the only one to have been reported in wildlife in France. In other French areas reporting unexplained cattle outbreaks, investigations of wildlife failed to detect any significant circulation of *M. bovis*. The fact that under similar epidemiologic conditions the disease was not transmitted effectively from domestic animals to wildlife may indicate that such a transmission is a rare event. Migration of animals to or from the Brotonne and Mauny forests is not very likely for geographic reasons. It is plausible that there are genetic factors that contribute to the resistance or susceptibility of these isolated populations of red deer and wild boar to tuberculosis; consanguineous mating may have contributed to the emergence of animals more susceptible to tuberculosis. Investigations of this issue may provide valuable clues for the development of wildlife management strategies to control this disease.

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LITERATURE CITED

Buddle, B. M., F. E. Aldwell, A. Pfeffer, and G. W. De Lisle. 1994. Experimental *Mycobacterium bovis* infection in the brushtail possum (*Trichosurus vulpecula*): Pathology, haematology and lymphocyte stimulation responses. Veterinary Microbiology 38: 241–254.

Coleman, J. D., and M. M. Cooke. 2001. *Mycobacterium bovis* infection in wildlife in New Zealand. Tuberculosis 81: 191–202.

Corner, L. A., R. H. Barrett, A. W. Lepper, V. Lewis, and C. W. Pearson. 1981. A survey of mycobacteriosis of feral pigs in the Northern Territory. Australian Veterinary Journal 57: 537–542.

De Lisle, G. W. 1994. Mycobacterial infections in pigs. Surveillance 21: 23–25.

Dohoo, I., M. Wayne, and H. Strynh. 2003. Veterinary epidemiologic research. AVC Inc., Charlottetown, Prince Edward Island, Canada, pp. 85–120.

Frothingham, R., and W. A. Meeker-O'Connell. 1998. Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. Microbiology 144: 1189–1196.

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

Haddad, N., A. Ostyn, C. Karoui, M. Masselot, M. F. Thorel, S. L. Hughes, J. Inwald, R. G. Hewinson, and B. Durand. 2001. Spoligotype

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diversity of *Mycobacterium bovis* strains isolated in France from 1979 to 2000. Journal of Clinical Microbiology 39: 3623–3632.

HÉNAULT, S., C. KAROUI, AND M. L. BOSCHIROLI. 2006. A PCR based method for tuberculosis detection in wildlife. *In* New diagnostic technology: Applications in animal health biological and controls. Developments in biologicals, P. Vannier and D. Espeseth (eds.). Karger, Basel, Switzerland, pp. 123–132.

KAMERBEEK, J., L. SCHOULS, A. KOLK, M. VAN AGTERVELD, D. VAN SOOLINGEN, S. KUIJPER, A. BUNSCHOTEN, H. MOLHUIZEN, R. SHAW, M. GOYAL, AND J. VAN EMBDEN. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. Journal of Clinical Microbiology 35: 907–914.

KENT, P. T., AND G. P. KUBICA. 1985. Public health mycobacteriology. A guide for the level III laboratory. Centers for Disease Control and Prevention, Atlanta, Georgia.

LUGTON, I. W., P. R. WILSON, R. S. MORRIS, AND G. NUGENT. 1998. Epidemiology and pathogenesis of *Mycobacterium bovis* infection of red deer (*Cervus elaphus*) in New Zealand. New Zealand Veterinary Journal 46: 147–156.

MACKINTOSH, C. G., G. W. DE LISLE, D. M. COLLINS, AND J. F. GRIFFIN. 2004. Mycobacterial diseases of deer. New Zealand Veterinary Journal 52: 163–174.

MORRIS, R. S., AND D. U. PFEIFFER. 1995. Directions and issues in bovine tuberculosis epidemiology and control in New Zealand. New Zealand Veterinary Journal 43: 256–265.

———, ———, AND R. JACKSON. 1994. The epidemiology of *Mycobacterium bovis* infections. Veterinary Microbiology 40: 153–177.

NUGENT, G., J. WHITFORD, AND N. YOUNG. 2002. Use of released pigs as sentinels for *Mycobacterium bovis*. Journal of Wildlife Diseases 38: 665–677.

O'BRIEN, S. M. SCHMITT, J. S. FIERKE, S. A. HOGLE, S. R. WINTERSTEIN, T. M. COOLEY, W. E. MORITZ, K. L. DIEGEL, S. D. FITZGERALD, D. E. BERRY, AND J. B. KANEENE. 2002. Epidemiology of *Mycobacterium bovis* in free-ranging white-tailed deer, Michigan, USA, 1995–2000. Preventive Veterinary Medicine 54: 47–63.

PALMER, M. V., W. R. WATERS, AND D. L. WHIPPLE. 2004. Shared feed as a means of deer-to-deer transmission of *Mycobacterium bovis*. Journal of Wildlife Diseases 40: 87–91.

PARRA, A., A. GARCIA, N. F. INGLIS, A. TATO, J. M. ALONSO, M. HERMOSO DE MENDOZA, J. HERMOSO DE MENDOZA, AND J. LARRASA. 2005. An epidemiological evaluation of *Mycobacterium bovis* infections in wild game animals of the Spanish Mediterranean ecosystem. Research in Veterinary Science 80: 140–146.

ROHONCZY, E. B., A. V. BALACHANDRAN, T. W. DUKES, J. B. PAYEUR, J. C. RHYAN, D. A. SAARI, T. L. WHITING, S. H. WILSON, AND J. L. JARNAGIN. 1996. A comparison of gross pathology, histopathology, and mycobacterial culture for the diagnosis of tuberculosis in elk (*Cervus elaphus*). Canadian Journal of Veterinary Research 60: 108–114.

SCHMITT, S. M., S. D. FITZGERALD, T. M. COOLEY, C. S. BRUNING-FANN, L. SULLIVAN, D. BERRY, T. CARLSON, R. B. MINNIS, J. B. PAYEUR, AND J. SIKARSKIE. 1997. Bovine tuberculosis in free-ranging white-tailed deer from Michigan. Journal of Wildlife Diseases 33: 749–758.

SIMPSON, V. R. 2002. Wild animals as reservoirs of infectious diseases in the UK. Veterinary Journal 163: 128–146.

SKUCE, R. A., T. P. MCCORRY, J. F. MCCARROLL, S. M. RORING, A. N. SCOTT, D. BRITTAIN, S. L. HUGHES, R. G. HEWINSON, AND S. D. NEILL. 2002. Discrimination of *Mycobacterium tuberculosis* complex bacteria using novel VNTR-PCR targets. Microbiology 148: 519–528.

VICENTE, J., U. HOFLE, J. M. GARRIDO, I. G. FERNANDEZ-DE-MERA, R. JUSTE, M. BARRAL, AND C. GORTAZAR. 2006. Wild boar and red deer display high prevalences of tuberculosis-like lesions in Spain. Veterinary Research 37: 107–119.

WAKELIN, C. A., AND O. T. CHURCHMAN. 1991. Prevalence of bovine tuberculosis in feral pigs in central Otago. Surveillance 18: 19–20.

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