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## Spatial association of *Mycobacterium bovis* infection in cattle and badgers at the pasture interface in an endemic area in France

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### ABSTRACT

Despite control and surveillance programmes, *Mycobacterium bovis*, the main aetiological agent of bovine tuberculosis (bTB), is still detected on cattle farms and in wildlife populations in France, especially in badgers in the French Côte-d'Or département. The aim of our study was to find out if infected badgers were trapped significantly closer to pastures of infected farms than non-infected badgers and, if so, to determine the most efficient distance around those pastures for badger trapping, particularly for surveillance purposes. We studied two subareas (southern and northern), chosen based on natural barriers to badger movements and according to the presence of pastures belonging to infected farms (POIFs) and infected or non-infected badgers. In each subarea, we computed the shortest distances  $D_0$  and  $D$  between badgers trapped a given year  $n$  between 2015 and 2019 ( $n = 59$  infected and  $n = 1535$  non-infected badgers for  $D_0$ ;  $n = 53$  infected and  $n = 1476$  non-infected badgers for  $D$ ) and POIFs designated as infected between the year  $n - 4$  and  $n + 1$  (respectively  $n = 373$  and  $n = 388$  POIFs).  $D_0$  was calculated without considering spoligotypes, while  $D$  was calculated considering the possible epidemiological link between infected badgers and POIFs by using bTB spoligotype information. Then, we computed the observed mean and median of the  $D_0$  and  $D$  distances and used a bootstrap analysis to test if infected badgers were found significantly closer to POIFs than non-infected badgers. We observed that infection of badgers was not independent of distance from POIF in both subareas but distances ( $D_0$  or  $D$ ) were different between the northern and southern subarea. In the northern subarea, which displays a mosaic landscape (mean and median  $D$  distances were respectively 612 m and 303 m for infected badgers), infected badgers indeed were trapped closer to POIFs, considering  $D_0$  and  $D$ . In the southern subarea, predominantly forested, infected badgers were significantly closer to POIFs than non-infected badgers when considering  $D_0$  but not for  $D$  (mean and median  $D$  distances were respectively 7148 m and 4831 m for infected badgers). These results will help to determine the most efficient distance from POIFs to trap badgers to determine their infection status in countryside landscapes. They also highlight the need to better understand the epidemiological systems at play in more forested landscapes where badgers may behave differently or other susceptible sympatric wild species might play a more important role in the circulation of *M. bovis*, both phenomena contributing to badger infection at greater distances from POIFs.

### 1. Introduction

Although France is officially free of bovine tuberculosis (bTB), the disease is still endemic in domestic animals in some regions, especially in cattle (Crozet et al., 2019; Delavenne et al., 2020; Guétin-Poirier

et al., 2020). Furthermore, *Mycobacterium bovis*, the main aetiological agent of bTB, is also still regularly detected in wildlife populations. For example, 286 cattle farm outbreaks were detected between 2015 and 2017, with a slow increase in farm prevalence, reaching 0.1% in 2017 (Delavenne et al., 2020). Surveillance and control measures targeting

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wildlife are now part of the French national plan for bTB control, including the 'Sylvatub' surveillance programme, which was launched in September 2011 (Rivière et al., 2013). This plan aims at detecting and monitoring *M. bovis* infection in wild species such as wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and European badger (*Meles meles*) populations, by means of both event-based and targeted surveillance strategies. This programme and related studies revealed that wildlife infection has always been detected in the vicinity of bTB cattle outbreaks (Payne et al., 2013; Richomme et al., 2013; Réveillaud et al., 2018). Between 2016 and 2017, within infected areas at the national level, the apparent infection rates were 5.14% in badgers and 2.37% in wild boars, and sporadic infections were detected in roe deer and red deer (Réveillaud et al., 2018).

Within the areas where *M. bovis* persists, the epidemiological system is thought to be complex, involving several species, both domestic and wild. Among the latter, badgers form colonies involving setts, display some specific diet characteristics (e.g. foraging for earthworms in the soil) and have the capacity to shed bacteria in urine and faeces (Corner et al., 2011; Payne, 2014; King et al., 2015), features that together suggest that they play a particular role in the *M. bovis* epidemiological system (Payne et al., 2016; Varela-Castro et al., 2021). *M. bovis* can survive several weeks in the soil (Allen et al., 2021; Barbier et al., 2017), making time-lagged transmission possible, based on indirect contacts. Unlike in the British Isles, the badger is so far not considered a long-term maintenance host (Payne, 2014) in bTB endemic areas in France but more as a spill over host (Fenton and Pedersen, 2005) able to transmit *M. bovis* infection to cattle. Indeed, even if a spill over host is not able to maintain an infection alone for a long-term period, it can be part of a multi-host system, which can act as a maintenance community (Haydon et al., 2002) and thus contribute to the local circulation of *M. bovis*. In a recent spatially explicit model, the effective reproduction number within the badger-cattle system was estimated to be 1.34 regarding *M. bovis* transmission, while those for within cattle and badger populations were below 1. These results indicated that neither cattle nor badger populations could be considered as separate reservoir hosts, while the *M. bovis* transmission could be self-sustained within the badger-cattle maintenance community (Bouchez-Zacria et al., 2023). In this context, infected badgers might be responsible for the infection of naïve cattle herds or reinfection of former infected herds. In the few studies conducted in France, badger home ranges (here, the maximum area badgers are able to cover during their tracking period) were estimated within a range from 0.90 km<sup>2</sup> to 2 km<sup>2</sup> in the Ardennes département<sup>1</sup> (Bodin, 2005) and from 0.27 km<sup>2</sup> to 3.9 km<sup>2</sup> in the Côte-d'Or département (Payne et al., 2014), with seasonal variability (larger in summer than in winter and intermediate in autumn and spring). In this latter département, badgers inhabiting more fragmented landscapes tended to use more the pastures than badgers whose setts were located in the woods, indicating an effect of landscape characteristics on the potential interactions between badgers and cattle (Payne, 2014). The maximum distance recorded within the home ranges can vary from 912 m to 3.5 km (Payne, 2014). A previous study conducted in a third bTB endemic area demonstrated the existence of concomitant infection zones between badgers and cattle (i.e. where both species have been found infected with the same *M. bovis* spoligotype) in a radius between 500 m and 1000 m around the main badger setts (Bouchez-Zacria et al., 2017). Hence all these observations lend credibility to a spatial association between *M. bovis* infection in cattle and in badgers within relatively short distances.

As part of the Sylvatub programme, badgers are trapped and tested for *M. bovis* surveillance and control purposes. Trapped animals are being culled for complete bTB diagnosis and as part of control actions.

Our study used data collected within the Sylvatub programme. Before 2019, they were trapped in the entire at-risk areas for bTB. An at-risk area is composed of an infected area (where infection has been detected in domestic and/or wild animals and where trapping is organised for surveillance and control purposes) and a buffer zone (where trapping was organised for surveillance purpose only). These areas are defined every year according to (i) the locations of pastures where infected cattle may have grazed, and (ii) infected badger locations (see Réveillaud et al., 2018 for details). Since 2019, badger trapping has only been conducted in infected areas. In buffer zones, *M. bovis* surveillance is now only based on the analysis of road-killed badgers. Nevertheless, the infected areas may still be very large in some French départements and coverage of the entire zone by trapping remains very difficult, both for logistical (e.g. the decreasing number of trappers) and ethical reasons. Therefore, to optimise control actions, trapping may be targeted to restricted zones where culling badgers is most relevant.

Evaluating the spatial relationship between infected badgers and cattle occupying pastures of infected farms is therefore important for targeting badger trapping and improving bTB control measures. Understanding the spatial relationship between cattle and badgers can also provide insight into the role of the latter in the *M. bovis* multi-host system. The objectives of the present study were twofold: 1) to determine whether infected badgers were significantly closer to pastures belonging to infected cattle farms than non-infected badgers; 2) if so, to determine the mean and median shortest distances between infected badgers and pastures of infected farms (POIFs), with the goal of defining a preferential distance for badger culling (in a surveillance and control context) around infected pastures. In order to take badger movements into consideration, we have defined subareas outside of which badgers could not move. For the first objective, in order to take into account the possibility of undetected infected animals, the spatial distribution of infected badgers in relation to pastures of infected farms was evaluated without taking spoligotype data into consideration, through a distance  $D_0$  between badgers and their closest POIF. For the second objective, we used spoligotype data to link infected badgers and POIFs and computed a distance  $D$ , between badgers and their closest POIF, provided that they shared the same spoligotype.

## 2. Materials and methods

### 2.1. Study area

The study was carried out in France in the Côte-d'Or département (French administrative division situated in the traditional Burgundy region, now part of the Burgundy-Franche-Comté region, north-eastern France). In this département, the Sylvatub programme has been implemented since 2012, with regular *M. bovis* detection in badgers, as well as in ungulates (Réveillaud et al., 2018). For example, from 2016 to 2017, 3.6% of the captured badgers (22/608) proved to be infected as were 1.8% of the wild boars sampled during the 2015–2016 and 2016–2017 hunting seasons (8/440) (Sylvatub data). In 2016 and 2017, badger density was estimated at  $4 \pm 1$  badgers per km<sup>2</sup> (adults and cubs) (Jacquier et al., 2021). During the 2015–2016 surveillance period, the percentage of infected cattle herds was 0.7%, with 11 infected farms (S. Girard, personal communication).

For the present study, the département was divided into four independent subareas, considering high-traffic roads as barriers to badger movements (Clarke et al., 1998; Frantz et al., 2010; Sleeman et al., 2008): a northern subarea (4449 km<sup>2</sup>), a southern subarea (735 km<sup>2</sup>), a south-eastern subarea (630 km<sup>2</sup>) and a south-western subarea (1609 km<sup>2</sup>). The study area was composed of the two subareas including pastures of cattle farms testing positive for bTB between 2011 and 2020, and badgers trapped and analysed between 2015 and 2020, i.e. the northern and the southern subareas. The south-eastern subarea did not contain any pastures of infected farms (POIFs) and the south-western subarea did not include any infected badgers; they were both

<sup>1</sup> A French département is a NUTS 3 division according to Eurostat NUTS classification <https://ec.europa.eu/eurostat/documents/345175/7451602/2021-NUTS-3-map-FR.pdf>

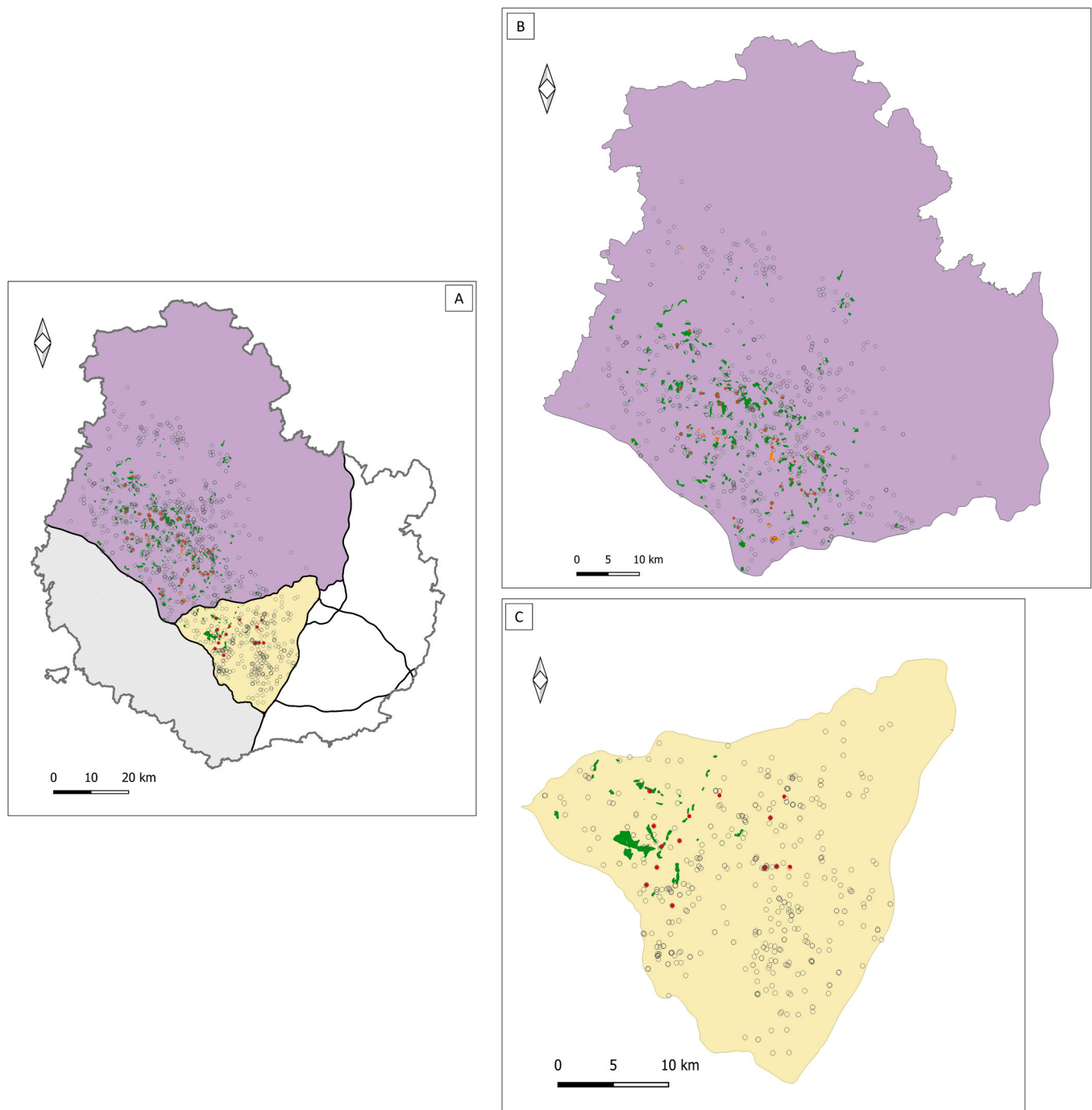
therefore excluded from the study (Fig. 1).

The northern subarea is a rolling countryside, with agricultural land (mostly pastures) alternating with woods in its southern sector, and a plateau with crop fields in the northern part. The southern subarea is predominantly covered by forests, and vineyards in its eastern sector, with only a relatively small area covered by pastures.

## 2.2. Badger data

Badger data used in our study were provided by the trapping of

badgers implemented in the Sylvatub national surveillance programme, led by the Ministry in charge of Agriculture. Badgers were trapped with stopped restraints set up near setts and culled by head shots. In order to limit the stress of captured badgers, traps were checked in the early morning (first hours after sunrise), the day after they were set up, in accordance with national trapping regulations. Every badger trapped was sent to the local veterinary laboratory. Analyses at the local laboratory consisted in post-mortem necropsy to detect bTB-like lesions (caseo-granulomas, mineralised nodules, or purulent abscesses) and systematic polymerase chain reaction (PCR) tests on pooled lymph node



**Fig. 1.** Spatial distribution of badgers (2015–2019) and pastures of infected farms (POIFs) (2011–2020) in the Côte-d'Or département study area (A: département and location of the study area; bold grey border: Côte-d'Or département border; purple area: northern study area; sand area: southern study area; black lines: roads; grey areas: south-eastern (SE, shaded line) and south-western (SW, shaded) areas (both excluded); red points: infected badgers; grey circles: non-infected badgers; green polygons: POIFs with identified spoligotype; orange polygons: POIFs without identified or partial spoligotype; B: northern subarea (legend identical to A); C: southern subarea (legend identical to A).

samples and on pooled tissues with bTB-like lesions. Bacterial culture and spoligotyping were performed on positive PCR samples. Laboratory testing methods are detailed in Réveillaud et al. (2018).

Spatial coordinates of trapped badgers have been collected consistently since 2015 in the study area. We therefore compiled 1535 *M. bovis* status results (infected or non-infected) of badgers trapped between 2015 and 2019 along with the spatial coordinates of the trapping spot. For infected animals, the year of infection and spoligotype were also known. Spoligotyping results were of three types: SB0120, SB0134 or not identified (Table 1).

### 2.3. Cattle data

In the study area, cattle over one year old, and from surveillance campaign 2017–2018 over 18 months, were tested yearly for bTB using intradermal comparative cervical tuberculin tests (ICCTT). Surveillance was also carried out on every slaughtered animal by national meat inspection services (macroscopical surveillance of bTB-like lesions). A farm was defined as bTB-infected according to national regulations, i.e. when at least one animal of the farm has been proven to be infected with *M. bovis* (by molecular diagnosis or by bacterial culture). Spoligotyping was also performed on every positive bovine sample.

Regarding POIFs, we initially surveyed 2276 temporary and permanent pastures. They were extracted from the ‘Relevé Parcelaire Graphique’ (RPG), which collects farmers’ declarations for the Common Agricultural Policy (CAP); and belonged to 112 infected farms detected bTB-positive from 2011 to 2020. For each POIF, the year of infection and the spoligotype were known. Spoligotyping results were of six types: SB0120, SB0134, both SB0120 and SB0134, SB0849, partial or not identified (Table 1).

### 2.4. Distance matrix computation

We computed the Euclidean distance (in metres) between each badger (with known spatial coordinates) and the centroid of POIFs (we considered that badgers entered the POIFs), under the condition that they belonged to the same subarea (whose boundaries were defined as barriers to badger movements). The shortest distance between a given badger and a POIF was named  $D_0$  or D since we followed two different approaches to calculate those distances. The distance  $D_0$  referred to the distances between infected badgers and the nearest POIF without taking into consideration the spoligotype (infected badgers and nearest POIF

**Table 1**

Distribution of spoligotypes among badgers (2015–2019) and pastures of infected farms (POIFs) (2011–2020) depending on subarea and before the computation of the distance matrices between badgers and their closest POIF.

	Location		
	Northern subarea	Southern subarea	Total area
<b>Number of badgers</b>			
Non-infected	980	496	1476
SB0120 <sup>a</sup>	33	7	40
SB0134 <sup>b</sup>	4	9	13
Not identified spoligotype	5*	1*	6*
Total	1022* (1017**)	513 ** (512**)	1535* (1529**)
<b>Number of POIFs</b>			
Not identified or partial spoligotype	143 * *	0	143 * *
SB0120 <sup>a</sup>	1559	4	1563
SB0120 <sup>a</sup> and SB0134 <sup>b</sup>	67	0	67
SB0134 <sup>b</sup>	311	172	483
SB0849 <sup>c</sup>	20 * *	0	20 **
Total	2100* (1937 **)	176*,**	2276* (2113 **)

\* :  $D_0$  analysis; \*\*: D analysis; a,b,c: deleted spacers of spoligotypes profiles with a: 3,9,16,39–43; b: 3–5,9,16,39–43; c: 3,5,9,16,39–43

may be of different spoligotypes). This generic approach was performed in order to use similar distances between infected and non-infected badgers to test the null hypothesis of independency between badger locations and POIFs. In contrast, the distance D was calculated differently for infected badgers since the nearest pasture had to be of the same spoligotype. This calculation was performed to investigate, for each subarea, the distances between infected badgers and POIFs with a plausible epidemiological link.

For each badger trapped and analysed in the year  $n$ , we selected the nearest POIF during the  $n - 4$  to  $n + 1$  period. Due to annual testing, test performance and non-exhaustive trapping, we could not exclude that transmission, regardless of the direction of transmission, had potentially occurred between a badger detected infected in year  $n$  and a farm detected infected four years before or one year later.

In order to compute the  $D_0$  distance matrix, all badgers and POIFs were considered, without taking into account spoligotype for infected badgers. Ultimately, 1535 badgers (Table 1) and 388 POIFs (Table 2) were included in the  $D_0$  distance matrix.

For the D distance matrix, we first excluded badgers without identified spoligotypes ( $n = 6$ ) since a plausible epidemiological link with a POIF could not be established (Table 1), as well as POIFs with SB0849 spoligotype ( $n = 20$ ) (this spoligotype was not shared with badgers) and POIFs without identified or partial spoligotype ( $n = 143$ ) (the possible transmission link between cattle and badgers was unprovable) (Table 1). For each non-infected badger, the closest POIF was the POIF regardless of its spoligotype. Ultimately, 1529 badgers (Table 1) and 373 POIFs (Table 2) were included in the D distance matrix.

### 2.5. Bootstrap analysis and Kolmogorov-Smirnov test

The null hypothesis was that infection of badgers is independent of distance from POIFs, that is to say, that there was no difference between the distance  $D_0$  of non-infected and infected badgers. To test this hypothesis, we used the difference in the means of  $D_0$  (or in the medians of  $D_0$ ) between non-infected and infected badgers. This bootstrap analysis was also performed for the D distance.

A bootstrap analysis was chosen to test the null hypothesis, given that our data were not independent. This type of analysis is also particularly appropriate when the studied variable is not distributed normally and the sample size is small (Davison and Kuonen, 2002). This was indeed the case in our study, where the number of infected badgers was low, and the distribution of the  $D_0$  and D distances did not follow a normal distribution (Supplementary Materials, Fig. S1).

In the dataset of badgers analysed for the study period (observed data), SB0120, SB0134 badgers and those with non-identified spoligotypes were classified as infected for the  $D_0$  bootstrap analysis, while SB0120 and SB0134 badgers (as badgers without identified spoligotype were excluded) were classified as infected for the D bootstrap analysis. The mean of the  $D_0$  (D) distance was calculated for infected and non-infected badgers, as well as the difference between the two values

**Table 2**

Distribution of POIFs included in the bootstrap analysis (N: northern subarea; S: southern subarea).

POIF spoligotype	Subareas					
	$D_0$ analysis			D analysis		
	N	S	Total	N	S	Total
SB0120	239	2	241	252	2	254
SB0134	48	47	95	49	47	96
SB0120-SB0134*	23		23	23	0	23
SB0849	7	0	7	-	-	-
Not identified/partial	22	0	22	-	-	-
Total	339	49	388	324	49	373

\* : several outbreaks occurred in farms related to POIFs, with different spoligotypes



(difference of the observed means). Similarly, the median of the  $D_0$  (D) distance was calculated for infected and non-infected badgers, and the difference between the two medians (difference of the observed medians).

The bootstrap analysis consisted of 10,000 permutations in which the  $D_0$  (D) distance was randomly reallocated to non-infected and infected badgers, keeping the same number of infected and non-infected badgers as in the observed dataset. For each permutation, we calculated the mean of the  $D_0$  (D) distance for non-infected and infected badgers and the difference between the two values. Similarly, for each permutation, we computed the median of the  $D_0$  (D) distance for infected and non-infected badgers and the difference between the two medians. Thus, we obtained the distribution, under the null hypothesis, of the difference in means of  $D_0$  (D) between non-infected and infected badgers from permutations, and of the difference in medians of  $D_0$  (D) between non-infected and infected badgers from permutations.

The significance of the difference in the means and medians of  $D_0$  (D) were determined by calculating a  $p$ -value for each of these differences. The  $p$ -value of the difference of the means (or medians) of  $D_0$  (D) was approximated by the proportion of permutations with a greater difference than the one observed. The difference was considered significant when the  $p$ -value was less than 5% ( $\alpha$  error).  $D_0$  and D bootstrap analysis were stratified by northern and southern subareas.

In addition, to compare the  $D_0$  (D) distance distribution in both subareas, we used a Kolmogorov-Smirnov test (Taylor and Emerson, 2011).

We used QGIS© software version 3.4 (QGIS Development Team, 2018) to divide the study area into subareas and R© software version 3.5.1 (R Core Team, 2018) for all other computations and analyses. Packages sf (Pebesma, Bivand, 2005) and dgof (Taylor and Emerson, 2011) were used respectively for spatial computations and Kolmogorov-Smirnov test.

### 3. Results

Among the 1535 badgers included in the  $D_0$  bootstrap analysis, 59 were infected, while 53 were infected among the 1529 badgers included in the D bootstrap analysis, with a great predominance of SB0120 in the northern subarea in both analysis (Table 1).

The distributions of the  $D_0$  and D distances were not normal and justified the use of a bootstrap approach (Supplementary Materials, Fig. S1). Considering the  $D_0$  distance in northern subarea, 75.8% of SB0120 infected badgers match the spoliotype of the nearest infected pasture but only 50% for SB0134 infected badgers. In the southern. In the southern subarea, none of SB0120 infected badgers match the spoliotype of the nearest infected pasture (there are only two SB0120 infected pastures), but 100% for the SB0134 infected badgers.

The mean and median of the observed  $D_0$  and D distances are presented in Table 3.

In the northern subarea, the distribution of the  $D_0$  and D distances suggested a difference between non-infected and infected badgers (Fig. 2A and Fig. 3A). The  $D_0$  and D bootstrap analysis showed that these differences between infected and non-infected badgers were significant, using the difference in means (respectively  $p = 0$  and  $p < 0.0001$ ) (see Supplementary Materials, respectively Fig. S3A and Fig.S4A) or the difference in medians (respectively  $p = 10^{-4}$  and  $p < 0.0001$ ) (see

Supplementary Materials, respectively Fig. S3C and Fig.S4C). The distribution of the difference in means of the  $D_0$  and D distances from the permutations was plotted (respectively highlight that in this infected zone, infection of badgers is not independent of distance from POIFs, Fig. 3B), showing that the observed differences in means of  $D_0$  and D were higher than the differences in means of  $D_0$  and D simulated under the null hypothesis (see Supplementary Materials for the test considering the difference of medians; Figs. S2A and S2C). The Kolmogorov-Smirnov test comparing the  $D_0$  distance distribution between non-infected and infected badgers was significant ( $p < 0.0001$ ), as well as for the D distance distribution ( $p < 0.0001$ ) indicating that the respective two distributions were different.

In the southern subarea, the distribution of the  $D_0$  distance suggested a difference between non-infected and infected badgers (Fig. 2C). The  $D_0$  bootstrap analysis showed that the difference between infected and non-infected badgers was significant, using the difference in means ( $p = 9.10^{-4}$ ) (see Supplementary Materials, Fig. S3B) or the difference in medians ( $p = 0.0051$ ) (see Supplementary Materials, Fig. S3D). The distribution of the difference in means of the  $D_0$  distance from the permutations was plotted (Fig. 2C), showing that the observed difference in means of  $D_0$  was higher than the difference in means of  $D_0$  simulated under the null hypothesis (see Supplementary Materials for the test considering the difference of medians; Fig. S2B). The Kolmogorov-Smirnov test comparing the  $D_0$  distance distribution between non-infected and infected badgers was significant ( $p = 0.03558$ ), indicating that the two distributions were different.

Therefore, for both the northern and southern subarea, infected badgers were not randomly distributed and were closer to POIFs than non-infected badgers.

In the southern subarea, the distribution of the D distance suggested a less obvious difference between non-infected and infected badgers (Fig. 3C). The bootstrap analysis showed that this difference was not significant, using the difference in means ( $p = 0.32$ ) (see Supplementary Materials, Fig. S4B). The distribution of the difference in means of the D distance from the permutations was plotted (Fig. 3D), showing that the observed difference in means of D was not higher than the difference in means of D simulated under the null hypothesis. The test considering the difference in medians of the D distance was not significant either ( $p = 0.07$ ) (see Supplementary Materials, Fig. S4D). Therefore, in the southern subarea, infected badgers were not significantly closer to POIFs than non-infected badgers, if spoliotypes were included in the analysis. The Kolmogorov-Smirnov test comparing the D distance distribution between non-infected and infected badgers was not significant ( $p = 0.15$ ) for the entire distribution: both distributions were equal.

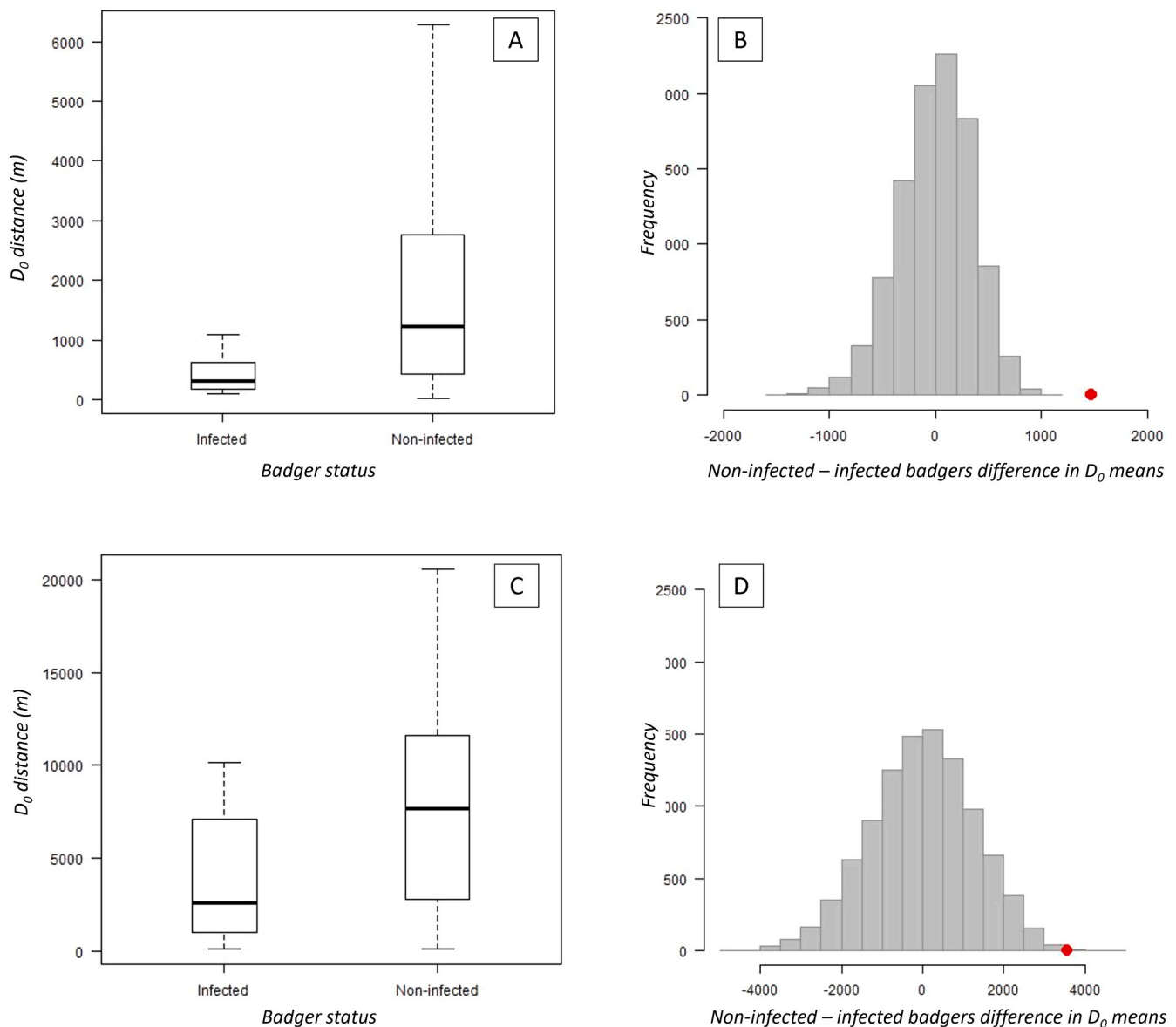
Regarding the more conservative calculation of the distance D between badgers and POIFs including spoliotype information, the mean and median of the observed D distance are presented in Table 3.

The  $D_0$  distance for infected badgers was significantly higher in the southern subarea than in the northern subarea (bootstrap test on the difference in mean and median of D within infected badgers: both  $p = 0$ ). The D distance for infected badgers was also significantly higher in the southern subarea than in the northern subarea (bootstrap test on the difference in mean and median of D within infected badgers: respectively  $p < 0.0001$  and  $p = 0.0002$ ).

When the northern subarea was considered separately, the mean D distance for infected badgers was 612 m and the median value was

**Table 3**  
Distribution of the  $D_0$  and D distances (in metres) depending on subarea (N: northern subarea; S: southern subarea;  $PI_{95\%}$ : 2.5% and 97.5% percentiles interval).

Subarea	Badger Status	$D_0$ distance (m)			D distance (m)		
		Mean	Median	$PI_{95\%}$	Mean	Median	$PI_{95\%}$
N	Infected	475	303	[91 – 1602]	612	303	[91 – 2261]
	Non-infected	1950	1226	[121 – 8712]	2006	1290	[122 – 9002]
S	Infected	4228	2606	[203 – 9669]	7148	4831	[200 – 16587]
	Non-infected	7800	7708	[388 – 18688]	7800	7708	[388 – 18688]



**Fig. 2.** Distribution of the  $D_0$  distances observed in non-infected and infected badgers (A: northern subarea; C: southern subarea) and of the difference in means in the bootstrap permutations ( $n = 10,000$ ) (B: northern subarea; D: southern subarea) (red point: observed value).

303 m (Table 3). When the southern subarea was considered separately, the mean D distance for infected badgers was 7148 m and the median value was 4831 m (Table 3).

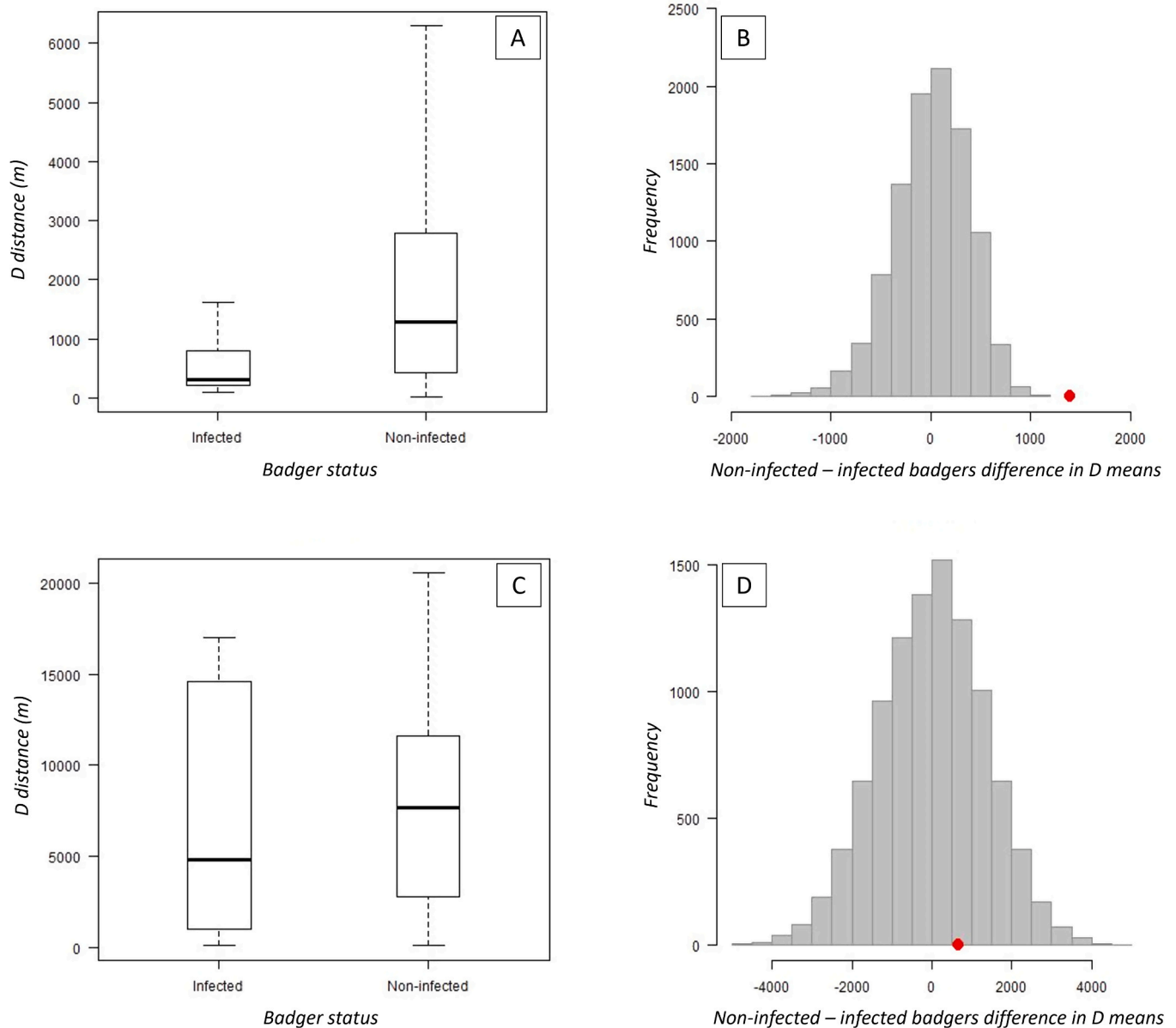
#### 4. Discussion

The question of the spatial distribution of infected badgers in relation with POIFs was addressed by studying the  $D_0$  distribution. The question of the operational distance at which to carry out trapping was addressed by studying the mean and median D distances for infected badgers. Indeed, these last distances reflect the epidemiological links between badgers and POIFs, by taking into account shared spoligotypes.

The distributions of the  $D_0$  and D distances were not normal and data not independent which justified the use of a bootstrap approach to test the difference in means (and medians) of  $D_0$  and D between non-infected and infected badgers from permutations.

The  $D_0$  distances differed significantly between infected and non-infected badgers for both northern and southern subareas. Thus, infection of badgers was dependent on the distance from POIFs (infected badgers were closer to POIFs than non-infected badgers). When

calculating  $D_0$ , we did not consider if badgers' spoligotypes matched the spoligotypes of the nearest POIF, in order to apply a similar definition of the shortest distance to POIF for both infected and non-infected badgers. Biologically, this approach relies on the possibility that a bovine might have infected a badger without being detected as infected, despite annual testing of the herds. This is supported by a French model related to cattle herds (from the 2000 s), which highlighted that in about 20% of infected herds (22% of beef herds and 17% of dairy herds) routine cull allowed the disappearance of bTB in case of introduction, with a median time lower than ten months (in median: six months in beef herds and seven months in dairy herds) (Bekara et al., 2014). Thus, cattle farms could transmit the pathogen to badgers, without any detection through herd testing. Moreover, a French badger-cattle model (related to populations of a south-western region of France) brought out that the *M. bovis* transmission from cattle farms to badger neighbouring groups was quite fast (six months in median after the infection of the cattle farm), and that the bTB would last for a long time in an infected badger group (six years in median before the Sylvatub programme implementation and three years after) (Bouchez-Zacria et al., 2023). Therefore, some badgers in our study could have been infected by an



**Fig. 3.** Distribution of the D distances observed in non-infected and infected badgers (A: northern subarea; C: southern subarea) and of the difference in means in the bootstrap permutations ( $n = 10,000$ ) (B: northern subarea; D: southern subarea) (red point: observed value).

undetected cattle herd, with no possibility for us to trace the epidemiological link. We can note that for the southern subarea, the effect of having or not the spoligotype in the analysis has more impact than for the northern area. When using the D distance instead of the  $D_0$  one, the bootstrap analysis did not allow to reject the null hypothesis for the southern area. This might be due to a more limited number of POIFs and infected badgers for this subarea. Thus, cattle detection holes and hidden epidemiological links (between cattle and badgers and/or with wild ungulates more abundant in this subarea) may have had more impact on this dataset.

When we adopted a more conservative approach with a calculation of the D distances which takes into consideration the spoligotypes, our results differed slightly for the northern subarea, but more significantly for the southern subarea. Regardless of the calculation, the mean and median of the D distance for infected badgers were significantly higher in the southern subarea than in the northern subarea.

The mean D distances for infected and non-infected badgers in this northern subarea were respectively 612 m and 2006 m and the median

D distances for infected and non-infected badgers were respectively 303 m and 1290 m. These results indicate that badgers from the northern subarea are more likely to be infected when living in the vicinity of POIFs than when living further away from these pastures. Although transmission may occur between badger individuals, as already demonstrated in other study fields (Crispell et al., 2019; Rossi et al., 2021; Tonder et al., 2021; Woodroffe et al., 2009), we showed that the spatial distribution of infection in the badger population in this subarea of Côte-d'Or remains correlated with the spatial distribution of infection in the cattle population through the interface of pastures belonging to infected farms within a five-year temporal window around the date of infection detection in cattle. This result is consistent with a study using data from the Randomised Badger Culling Trial (RBCT) in England (Woodroffe et al., 2005) which finds that *M. bovis* infections in badgers and cattle were spatially associated at a scale of 1–2 km. Based on the same dataset (RBCT), Tonder et al. (2021) conducted a phylogenetic study on field isolates and identified transmission clusters were highly localised geographically suggesting limited spatial



dispersion of the infection. In addition, they demonstrated that within badgers, transmission events were around five times higher than between badger to cattle or cattle to badger events. Nevertheless, they noted limitations in their methodology, which did not allow to draw firm conclusions on transmission dynamics between the species. In our northern subarea, the transmission dynamic might be different, in relation with differences in density and social structure of the badger population, (badger density being lower in France compared to England) and our findings suggests that interspecies transmission between cattle and badgers may be an important driver of the local persistence of the bTB infection. This could support the hypothesis of badgers being a spillover and not a maintenance host in our context, and/or, of a limited transmission by the badger population outside an initial cluster of infection. Further studies (including phylogenetic studies) would be needed to investigate the contribution of intraspecies vs. interspecies transmission in the *M. bovis* dynamics.

In the southern subarea, we found that the D distances for infected badgers were higher. The mean and median D distances for infected badgers were respectively 7148 m and 4831 m, values that were significantly higher than those of the northern subarea. Two non-exclusive hypotheses may be given. Firstly, the environment is different between the two areas and distances to cover to reach pastures where badgers can feed are higher. The southern subarea is mostly made of forest compared to the northern subarea, which displays a mosaic landscape with less forest and more agricultural land and the number of POIFs in this subarea was much lower than in the northern subarea (with a 15/100 ratio). As we observed that infected badger distribution was dependant on the POIFs spatial distribution ( $D_0$  bootstrap analysis), we could not exclude that badgers might cover higher distances in this subarea than typically observed in Europe (estimated at 1.7 km on average, with rare long distances of 22.1 km (Byrne et al., 2014)). In Côte-d'Or, a previous study has shown that badger movements could range from 912 m to 3.5 km around their setts (Payne, 2014). A second hypothesis might be that in this subarea, the multi-host system has a different composition: fewer cattle and more wild ungulates (wild boar and deer). Therefore, other susceptible sympatric wild species might play a more important role in the circulation of *M. bovis*, by covering the distances that might not be attributed to badger movements. For example, the average distance travelled daily by wild boars had been estimated at  $7.2 \pm 0.35$  km (Lemel et al., 2003). Regarding red deer, extreme distances of nearly 20 km have been reported in different studies including one in Côte d'Or (Payne, 2014 and Jarnemo, 2011). Such movements could therefore allow these ungulates to play a role in bTB transmission to badgers over long distances. Moreover, as part of the Sylvatub surveillance programme, both wild boars and deer are regularly detected bTB-positive in this zone, and recent serological investigations on the wild boar population showed a higher percentage of seropositive animals in the southern subarea compared to the northern subarea (Sylvatub programme). Therefore, wild ungulates could be a source of infection for badgers in this area. The epidemiological system at play in the southern zone might thus involve a more complex multi-host system, involving mostly wildlife, than in the northern zone (mainly composed of cattle and badgers). Further studies on the multi-host system are necessary to better apprehend this complexity.

Thus, it is important to consider the natural and epidemiological local environment to set up an optimal distance from POIFs to trap badgers. If compared with other endemic areas in France, the northern subarea of Côte-d'Or is more representative of bTB-infected areas involving badgers and cattle. In this context, to detect *M. bovis* infection and for disease control purposes, it would be more efficient to trap badgers near POIFs. Therefore, a distance between 300 m and 620 m (using respectively the median – less sensitive to the extreme values of the distribution and giving an estimate of the most effective D distance – and mean distances – to include the possibility of higher D distances – calculated for this northern subarea) can be chosen in an area where the landscape and the badger-cattle interface are similar to what is observed

in the northern subarea. This would also be in accordance with previous studies in France in Côte-d'Or where tracking badgers showed that those whose setts were located in forests, at more than 300 m from the edge of a pasture, visited pastures less frequently than badgers whose setts were closer (Payne, 2014).

This distance is also of particular interest for areas where disease in wildlife has not been detected yet. In those areas (called “prospection zones” in the Sylvatub programme), where bTB has been detected only in cattle, badger surveillance is organised for one to three years around the pastures of infected farms to determine if the infection has spilled over into the wildlife compartment. In such prospection zones, it is essential to optimise the likelihood of detecting the disease if present, by concentrating the trapping effort to where badgers are most likely to be found infected. Based on the results of the present study, we recommend that infection status of wildlife species within prospection zones be determined by analysing badgers trapped in close vicinity of POIFs, especially in mosaic-type landscapes.

On the other hand, in areas where bTB infection has been detected in the badger population for several years, trapping at greater distances from POIFs may still be justified because the multi-host system might involve species other than cattle in the contamination of the badger population, because of possible under-detection in cattle or badger populations or because badgers may be forced to cover larger distances to access pasture in some types of landscape. Furthermore, the strategy of reactive culling alone on a restricted area has demonstrated variable and sometimes limited results in reducing bTB incidence in bovine herds in the UK (Brunton et al., 2017; Downs et al., 2019; Vial and Donnelly, 2012). In those areas, although trapping in the vicinity of POIFs is still a priority (because the spatial distribution of infected badgers is still correlated to the spatial distribution of POIFs), trapping should probably be extended beyond the 300–620 m distance from the POIFs.

In the present study, it was not possible to infer the direction of the transmission of infection. For example, a badger detected as infected in year  $n$  may have been infected on a pasture during the years preceding its trapping (hence the choice to consider four years prior to detection in the badger). However, cattle (captured by POIF) detected in year  $n + 1$  may have contaminated this badger or it may have been contaminated by the badger before its trapping. However, the rhythm and quality of tests (annual testing, test performance) and non-exhaustive trapping led us to consider a period for POIFs extending from four years prior to one year after badger trapping. Recent studies using whole-genome sequencing exhibited contrasting results, suggesting that the directionality of transmission may vary according to the geographical and epidemiological contexts (Crispell et al., 2019 and Rossi et al., 2021). Our study with contrasting results between the subareas highlights the complexity of *M. bovis* circulation in multi-host systems and the need for studies at a small landscape scale to better understand the epidemiological mechanisms that are specific to each multi-host system situation.

## 5. Conclusion

In landscapes favourable to a badger-cattle epidemiological system, as in the northern part of Côte-d'Or, infected badgers appear to live closer to pastures of infected farms than non-infected badgers, with distances of 300 m in median and 620 m in mean between pastures and infected badgers. We suggest that these distances could be used to define an efficient badger trapping buffer zone around pastures of infected farms in similar settings. Nonetheless, in places where epidemiological links are less obvious between POIFs and infected badgers, complementary studies on the diversity of the *M. bovis* transmission should be implemented to avoid undetected infection in the different domestic and wild compartments.

## Declaration of Competing Interest

The authors declare that the research was conducted in the absence

of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.prevetmed.2023.106044](https://doi.org/10.1016/j.prevetmed.2023.106044).

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