Assessment of bovine tuberculosis risk factors based on nationwide molecular epidemiology

Running title: molecular typing and bovine tuberculosis epidemiology

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Abstract

This assessment aimed to elaborate a statistical nationwide model to analyze the space-time dynamics of bovine tuberculosis in search of potential risk factors that could be used to better target surveillance measures. A database comprising *Mycobacterium bovis* molecular profiles from all isolates of Belgian outbreaks during the 1995-2006 period (N=415) allowed the identification of a predominant spoligotype (SB0162). Various databases compiling 49 parameters to be tested were queried using a multiple stepwise logistic regression to assess bovine tuberculosis risk factors. Two isolate datasets were analyzed: the first included all *Mycobacterium bovis* isolates, while the second only included data related to SB0162 type strain. When including all *Mycobacterium bovis* isolates in the model, several risk factors were identified: history of bovine tuberculosis in the herd ($P < 0.001$), proximity of an outbreak ($P < 0.001$), cattle density ($P < 0.001$) and annual amplitude of mean middle-infrared temperature ($P < 0.007$). The approach restricted to the predominant SB0162 type strain additionally highlighted the proportion of movements from an infected area during the current year as a main risk factor ($P = 0.007$). This study identified several risk factors for bovine tuberculosis in cattle, highlighted the usefulness of molecular typing in the study of bovine tuberculosis epidemiology and suggests a difference of behaviour for the predominant type strain. It also emphasizes the role of animals' movements in the transmission of the disease and supports the importance of controlling trade movements.

Introduction

Despite significant historical efforts and the implementation of eradication plans, bovine tuberculosis (bTB) remains a preoccupant issue in the European Union, with some Member States facing recently a re-emergence of the disease (10). Some countries succeeded in biologically eradicating bTB after implementing control measures, while others, declared as Officially Tuberculosis-Free (OTF), still notify outbreaks every year, despite ongoing eradication and control programs (10). Belgium was declared OTF in 2003, yet, 5 to 10 outbreaks are notified every year (12). In 2008, an increase in the number of reported outbreaks was noticed (12), as shown in Figure 1.

Numerous risk factors for bTB have been identified in cattle around the world. These risk factors include a variety of parameters in relation to wildlife, cattle contacts, movements, density of animals, etc. (reviewed in (20)) but number of studies lack standardization. Furthermore, bTB transmission cycles underlying the failure to eradicate *Mycobacterium bovis (M. bovis)* in cattle in some areas remain poorly understood, and several transmission hypotheses have been formulated: inadequate control measures, agro-environmental factors, latency, wildlife reservoirs and movements of infected animals (15). Partly because bTB control programs are an economical burden, national animal health authorities are considering downscaling current control measures, e.g. cancelling testing at purchase and reducing herd testing. Nevertheless, animal movements were shown to be a risk factor in other countries such as the United Kingdom (UK) (15, 16). Before applying these reductive measures, it therefore seems appropriate to investigate the true risk represented by animal movements in the country.

A database including all *M. bovis* isolates grown from outbreaks reported between 1995 and 2006 in Belgium was compiled. This database was instrumental in analyzing bTB dynamics

70 in Belgium during the 1995-2006 period. A full literature review for bTB risk factors allowed
71 identifying several potential risk factors to be tested in Belgium (20). A statistical model
72 initially developed on the basis of data collected in the UK (15) was then adapted to the
73 Belgian dataset in order to test these potential risk factors.

74 In addition, recent studies focusing on *M. bovis* strains isolated in cattle and badgers from the
75 UK confirmed the limited number of strains circulating in the UK, even though the bTB herd
76 prevalence is elevated (14, 38). On the other hand, the situation in Belgium is totally opposite:
77 there is a wide diversity of co-circulating strains, with one predominating, and the herd
78 prevalence is under 0.1% in the cattle population (12). It was thus decided to follow two
79 approaches: one including all strains isolated in the country during the period of interest (1995
80 – 2006), and the other one focusing on the predominant strain type, in order to possibly
81 highlight a difference in behaviour.

82 This molecular epidemiology approach, never carried out so far in Belgium, is valuable for
83 health authorities in order to re-assess and adapt current control measures applicable for the
84 surveillance of bTB and to challenge a possible reduction in herd and individual testing.

85 **Materials and Methods**

86 ***A. Database compilation***

87 A literature review of bTB risk factors allowed the identification of several parameters to be
88 tested as possible risk factors. These parameters were called predictors. All predictors used
89 were derived from the databases described below. They were all compiled into a unique
90 database. The analysis of bTB dynamics was bi-dimensional. The temporal reference was the
91 year, and the spatial unit was defined as follows: the territory was divided into 5 km by 5 km

92 cells, identified thanks to their *X* and *Y* Lambert coordinates; these cells were named pixels
93 (http://users.skynet.be/belgique/belgica.zip).

94 A database of all *M. bovis* isolates grown from bTB suspect sampled organs, at the Belgian
95 national reference laboratory for bTB between the 1st of January 1995 and the 31st of
96 December 2006, was the starting point of the analysis. Sampling was performed at the
97 slaughterhouse when suspect lesions of bTB were observed, according to the European
98 legislation (11). Once the presence of *M. bovis* was confirmed, molecular typing tools allowed
99 to individually genotype each strain. Three techniques were used in parallel to identify the
100 strain type: spoligotyping, IS6110-RFLP (Restriction Fragment Length Polymorphism –
101 IS6110), and MIRU (Mycobacterial Interspersed Repetitive Unit) - VNTR (Variable-Number
102 Tandem-Repeat). These techniques have been widely used for the identification of *M. bovis*
103 strains (9, 31) and were combined in the study area (1), where IS6110-RFLP proves to
104 improve the discriminatory power of MIRU-VNTR, given that 48% of the area isolates
105 display 8 or more copies of IS6110 (1). Isolates found in Belgium between 1995 and 2006
106 have been previously classified into 12 lineages, according to their combined
107 RFLP/VNTR/spoligotype molecular profiles, which allowed to identify a specific strain type
108 characterised by its unique SB0162 spoligotype as being predominant. SB0162 was identified
109 in 27% (N = 112) of all isolates (N = 415) (following the international nomenclature
110 developed by www.Mbovis.org) (35).

111 Other databases were released by the Federal Agency for the Safety of the Food Chain
112 (FASFC): a complete list of all registered cattle herds of the country as well as the annual
113 census of all herds (the number of animals per herd, as defined on the 31st of December of
114 each year, data available from 2000 and after) were made available. Cattle movement data

115 that took place between 1995 and 2006 were extracted from the National Cattle Tracing
116 System (SANITEL).

117 The Nature and Forest Division (NFD) provided data for several wildlife species. Annual
118 estimated populations of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boar
119 (*Sus scrofa*), fallow deer (*Dama dama*) and mouflons (*Ovis orientalis*) were included in the
120 model.

121 Land cover data were also part of the model. Different types of land cover were considered:
122 pasture, crops, forest, humid areas, urban areas and other vegetation. The length of forest-
123 pasture edge was also available for analysis and was defined as a specific number of meters
124 per pixel: the more important the length of forest-pasture edge, the greater the risk of potential
125 contact was assumed between wildlife and cattle in pasture.

126 Remotely sensed data for several bioclimatic indicators were used as bioclimatic data. The
127 collection of these data has been previously described (19). Altitude was included in the
128 model as well.

129 ***B. Risk Predictors***

130 Predictors were converted for each pixel, per year, to include biological, demographic,
131 climatic and topographic variables, such as distance to the centre of a bTB outbreak pixel,
132 densities of wildlife populations, eco-climatic data, land cover, movements and density of
133 cattle, all compiled and re-sampled at the 5 km resolution.

134 The predictor named 'disease persistence' (PBTB, antecedent of bTB) was included in the
135 model as follows: for each pixel, and for each year of the period, it was specified if bTB was
136 present or not. A note of 0 was allotted if no bTB outbreak had been registered in the pixel in
137 the previous year, while a note of 1 meant the presence of bTB in the pixel.

In case of the presence of bTB, *M. bovis* strain was specified. Cattle populations were converted in order to include 'density of cattle' per pixel in the model. Data concerning wild animal populations were originally available at the district level; they were further transformed and converted to be available at the pixel level under the name 'density of wild species'. Regarding land cover predictors, data included in the model were the percentage of occupation within the pixel for the different types of vegetation.

Raw data extracted from the National Cattle Tracing System (SANITEL) were preliminarily transformed before inclusion in the model, as follows. Each movement was referenced with two data: the first data was the pixel of location from which the animal moved (pixel off) and the other one for the pixel of location to which the animal moved (pixel on). Both data had to be paired and were coupled to three variables: the total number of cattle head movements into a pixel, the total number of movements from an outbreak pixel, and the resulting proportion of movements that originated from outbreak pixels. These three variables were added to the model. Movement data were then analyzed in two ways. First we investigated the impact of movements that were registered the year before the eventual occurrence of bTB in a given pixel. Second, we assessed the impact of movements taking place during the year of occurrence of bTB outbreaks in a given pixel. A total of 49 predictors were considered, as summarized in Appendix 1.

C. Statistical analyses

A stepwise multiple logistic regression analysis was applied to data in order to investigate the potential association between bTB occurrence and the predictors. This model was originally created by Gilbert and collaborators to assess the importance of animal movements in the transmission of bTB in Great Britain (15); this model was further adapted to the peculiar situation of the study area and to include the molecular biology aspect. A unique multi-annual

database including all the information regarding the 49 predictors per pixel and per year was built. For each year and each pixel, the absence or presence of bTB was specified. First, the following predictors were entered in the model: PBTB (status of bTB in the previous year) and short-distance spread (number of infected pixels in the previous year in a doughnut-shaped window 5 km in radius). These two predictors were shown to have a significant impact on the presence of bTB in the pixel. Then other variables were added to the model using a standard-entry stepwise procedure. The model was restricted to predictors with the highest predictive power, and only those presenting more than 1% of log-likelihood change after removal were retained. In other words, these best predictors were systematically tested with the others families of predictors. Finally, all the predictors showing a significant effect were tested together. The 49 predictors could not be entered all-together in the model at the same time because some of them were correlated (altitude, densities of wild species and forests surfaces), any significant effect could thus have been masked. A predictor was considered as being a significant risk factor when presenting a positive or a negative relationship with the presence of bTB ($P<0.05$).

The model was applied in two stages. The first stage included all *M. bovis* strains identified in Belgium during the period of concern. The second stage focused on the predominating type strains (SB0162), isolated in the country between 1995 and 2006 (35).

The whole statistical process was carried out with R software (29).

Results

A. All Mycobacterium bovis strains

The predictors presenting a significant relationship from the analysis of all bTB isolates (N = 415) are summarized in Table I. Two predictors were positively and significantly related to

185 the presence of bTB in a pixel: persistence of bTB (PBTB) and cattle density (BOV03). The
186 proximity with the centre of an infected pixel (Logtb5km) showed a negative relationship
187 with bTB, which means the closer a herd was from the centre of an infected pixel, the greater
188 the risk of being infected. These three predictors exhibiting the highest level of significance
189 were tested with each of the other families of predictors (movements, wildlife, bio-climatic
190 and land cover) in a backward selection approach. At each step, the variable with the lowest
191 Wald statistics value was discarded. Finally, all predictors presenting a significant relationship
192 with the presence of bTB were kept. Red deer and roe deer densities presented a significantly
193 negative relationship with the presence of bTB, as well as forest density per pixel, which
194 would suggest that, to date, no wildlife reservoir of bTB exists in Belgium.
195 The backward selection approach involving bio-climatic predictors as well as altitude
196 revealed that annual amplitude of mean middle-infrared (MIR) temperature presented a
197 positive relationship with the presence of bTB. On the other hand, the bi-annual amplitude of
198 mean MIR temperature, the normalized difference vegetation index (NDVI) phase of annual
199 cycle and altitude showed a negative relationship with the risk of bTB.

200 Once all the families of predictors had been tested separately with the three best predictors,
201 the variables presenting a significant effect were all tested together, as illustrated in table II.
202 Some predictors then lost their significant effect mostly because of co-linearity: red deer and
203 roe deer densities, percentage of forest cover per pixel and altitude.

204 ***B. Predominant Mycobacterium bovis spoligotype (SB0162)***

205 Only *M. bovis* isolates characterized as SB0162 strain types were included in the second step
206 of the model (N = 112). All results of this approach are presented in Table III. Persistence of
207 bTB (PBTB) presented a positive significant relationship, while cattle density showed no
208 relationship with the presence of bTB. The distance to the centre of an infected pixel

(Logtb5km) showed a negative relationship with bTB. Only these two variables were thus tested with the other families of predictors (backward selection method). The proportion of movements originating from infected pixels during the current year presented a significant relationship with the presence of bTB, as well as crop surface. Regarding bio-climatic variables, the annual amplitude of MIR temperature presented a significant effect on the risk of bTB occurrence. As when all *M. bovis* strains were included in the model, the bi-annual amplitude of mean MIR temperature, and the NDVI phase of annual cycle showed a significant negative relationship with the risk of bTB (Table III).

After testing all the families of predictors separately, a model including all variables significantly related to the presence of bTB was tested. As for the all *M. bovis* strains-approach, several predictors lost their significant effect. The only predictors showing a significant relationship were then the proportion of movements originating from infected pixels during the current year (positive relationship) and the bi-annual amplitude of mean MIR temperature (Table IV) (negative relationship).

Discussion

The model allowed to highlight several factors correlated with the presence of bTB nationwide (e.g., Belgium) and is the first study of that kind. A first approach considered all the strains isolated between 1995 and 2006, while a second approach focused on the predominant strain type characterised by its SB0162 spoligotype, most frequently isolated in Belgium over the past 13 years (35).

A history of bTB in a given pixel was shown to represent a significant risk factor for the presence of bTB, both in the all-inclusive and predominant strain-restricted approaches. This observation confirms the results of previous studies carried out in other countries. Indeed,

British groups demonstrated that bTB outbreaks occur in a repeated way in the same areas (36). It is likely that the source of infection has not been cleared and/or that permanent factors would make these areas particularly prone to the re-emergence of bTB.

The proximity of an infected pixel turned out to be a significant risk factor for bTB as well. The greater the distance to the centre of an infected pixel is, the lower the risk of infection. This was previously observed in the Republic of Ireland (18). In their study, Griffin and collaborators demonstrated that, in a short period of time, bTB outbreaks affect most frequently several herds at the same time rather than a sole herd, because the contiguity with other herds under restriction was a risk factor. Another study carried out in the same country and including 215 dairy herds showed the neighbouring with an infected herd was associated with an outbreak in a particular herd. Nevertheless, a bTB infection confirmed in adjacent herds could point to a common source of infection (8). North American scientists highlighted the importance of contacts between animals over fences as a particular risk factor for the transmission of *M. bovis* between infected and healthy animals (22, 25). Thus, the results observed in a low prevalence situation seem to confirm what was observed in areas where bTB prevalence is high.

Density of cattle is a significant risk factor for bTB in Belgium. In our study, this predictor was identified as a significant risk factor in the first approach, including all *M. bovis* isolates, but not when the statistical model was applied to SB0162 only. Intensive farming is a risk by itself because of the closer proximity of animals and thus increased contacts and interactions between them. Airborne transmission is indeed the principle route of infection in cattle (13). The higher the density of animals, the higher the probability of close contacts between them. The highest incidence of bTB is generally observed in areas where intensive farming is practiced (5). The trends in dairy cattle are going towards intensification in industrialized

256 countries, which means fewer, much bigger herds, and as a result, increased contacts between
257 animals and an increasing risk of bTB transmission (34). Under intensive conditions,
258 aerogenic transmission of *M. bovis* prevails (23).

259 Contrarily to what has been observed elsewhere, animal movements from an outbreak to
260 another herd were not shown to be a significant risk factor when all *M. bovis* strains were
261 included in the statistical model. The low rate of outbreaks observed every year did not permit
262 to highlight this risk factor in the country, contrarily to the studies carried out by Gilbert and
263 collaborators in Great Britain (15). On the other hand, it is difficult to determine whether the
264 differences between both countries can be explained by differences in the control of cattle
265 movements or by the level of prevalence. A study focusing on the analysis of cattle
266 movements between 1985 and 2003 in the UK relied on molecular typing to identify most
267 outbreaks reported in the North-East of England between 2002 and 2004 (16). Animal
268 movements had a major impact if animals were moved from a bTB endemic zone to a bTB
269 free-area. The second approach including predominating strain type SB0162 identified the
270 proportion of movements from infected pixels during the current year as a significant risk
271 factor.

272 Several wild species play an important role in the transmission of *M. bovis* to cattle. It is the
273 case for badgers in the United Kingdom and in the Republic of Ireland (4, 8, 17) and for
274 brush-tail possums in New Zealand (24). Deer infected with *M. bovis* were discovered in
275 North America (22), in the UK (6, 7), in the Republic of Ireland (28), in Spain (2) and in
276 France (40). *M. bovis* has frequently been isolated in wild boar in Western Europe, especially
277 in France, Spain and Italy (26, 32, 40). Even when *M. bovis* is not yet isolated from wildlife,
278 this risk must not be dismissed. The influence of wildlife densities on the emergence of bTB
279 outbreaks in Belgium was thus tested. In our study, nevertheless, no relationship could be

observed between the densities of the main wild species tested (roe deer, red deer, wild boar and incidentally fallow deer and mouflons, mainly present in Belgium parks and domestic herds), and the presence of bTB suggesting once more they do not represent a risk for cattle contamination as *M. bovis* is probably not circulating in wildlife species to date. On the other hand, the presence of bTB presented a negative relationship with wildlife population densities, and the same effect was observed for land covered by forests. It is most likely that both observations are linked, as these two variables correlate. One should recall that data on wildlife species were only available for relatively large administrative units, hence the lack of apparent statistically significant relationship could be caused by the lack of high resolution data, and those results should be interpreted cautiously.

The analysis of the SB0162 type strain data identified the proportion of a pixel occupied by crops as a significant risk factor. A hypothesis to explain this observation could be that farms are concentrated around culture areas, for the supply in fodder.

Several bio-climatic factors happened to appear as significant risk factors for the emergence of bTB. The annual amplitude of temperature on the earth surface would be a risk factor, as shown in both approaches (all strains *vs*. predominating strain). Climate indeed influences the survival of *M. bovis* in the environment (27). The environmental survival of *M. bovis* would be inversely proportional to mean daily temperatures, as suggested previously in New Zealand (21). Temperatures just above 0°C coupled with a strong hygrometry are in favour of *M. bovis* survival (3). Tanner and Michel also observed a longer survival of *M. bovis* in faeces in the winter and under moist conditions, in the Kruger National Park, South Africa (33). Nevertheless, scientific opinions diverge regarding the importance the environment plays in the survival of *M. bovis*, as well as all the survival times suggested by the different studies that focused on this aspect. Some authors suggest the survival times of infective doses of *M.*

304 *bovis* on fomites are relatively short under natural conditions (24). Older studies described
305 longer survival times: *M. bovis* mixed artificially with cow faeces and exposed on pasture
306 land survived at least 35 weeks in the winter, 28 weeks in autumn, and up to 14 weeks in the
307 summer, in southern England (Williams and Hoy, 1930, cited by (41)). In northern Europe,
308 *M. bovis* mixed with organic matter survived 22-47 weeks when exposed to sunlight at 24-
309 34°C, but up to 104 weeks if buried 5 cm below the surface of shaded soil (Genov, 1965,
310 cited by (41)). More recently, Young and collaborators suggested *M. bovis* BCG remains
311 viable in soil for more than 15 months (39). Many studies focusing on survival times of *M.*
312 *bovis* in the environment reached their conclusions under experimental conditions. In 1997,
313 scientists who worked on data collected in England and Wales suggested that bTB occurrence
314 was linked to the seasonality and to climatic changes from one year to another (37). It is
315 constantly reported that temperatures just above 0°C and a strong hygrometry are in favour of
316 *M. bovis* survival, and these conditions are frequent in North-Western Europe in the
317 wintertime. The potential impact of climate change on *M. bovis* survival and on the
318 occurrence of bTB outbreaks should gain further attention, as scientific opinions still diverge
319 to date. Scientists from the UK recently evoked the potential role of free-living protozoa as an
320 environmental reservoir of *M. bovis,* which could contribute to the environmental persistence
321 of the mycobacteria (30). This possibility should not be neglected either.

322 The risk of bTB seems to decrease as altitude increases. The highest areas of the country are
323 located in the South and East of Belgium, regions where few or even no outbreaks were
324 reported to date. This trend could be put in relation with forests, mostly located in the same
325 regions.

326 This is the first nationwide study analyzing bTB risk factors combining three typing
327 techniques. The statistical analysis of relationships between bTB outbreaks and *M. bovis*

strain types allowed the identification of some risk factors: antecedents of bTB in a herd or in a small area, the proximity with an outbreak and cattle density. These observations should pave the way to an increased vigilance in matter of epidemiological investigations and eradication of ongoing outbreaks. Animal movements from infected areas were shown to be at risk for the predominant *M. bovis* SB0162 strain type circulating in Belgium, it is thus essential not to slacken vigilance in the control of movements and skin testing at purchase. Wildlife does not seem to represent a risk in Belgium to date, but the epidemiological surveillance is crucial within sight of the situation in neighbouring countries such as France or the UK. Studies focusing more specifically on the role of environment and climate in the persistence of *M. bovis* should be undertaken as well. In addition, the results of this study also suggest a difference of behaviour for the SB0162 type strains, underlying the importance of molecular epidemiology to investigate potential differences of virulence according to the strain.

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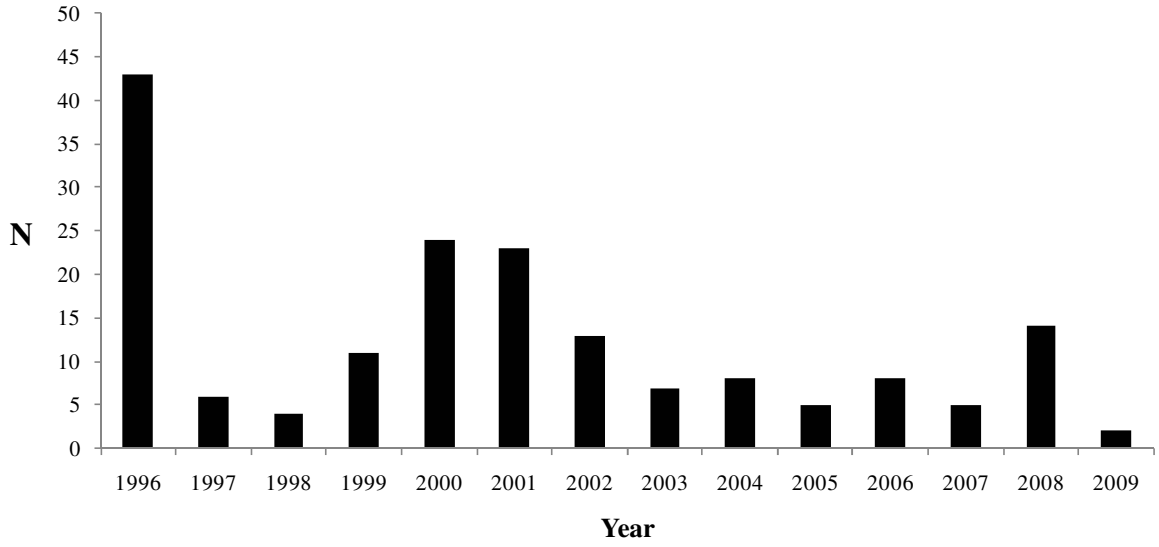
Figure 1



Trends followed by the number of bTB outbreaks (N) in Belgium between 1995 and 2009, as reported to the World Animal Health Organization; adapted from (12). The situation worsened in 2008 as the number of outbreaks almost tripled compared to the year 2007.

Table I: Summary of statistics for the predictors presenting a significant relationship with the occurrence of bTB (all isolates) **–** Multivariate analysis: backward selection approach

Predictor	**Estimation**	**Standard error**	***Z* value**	***P* value**	**Significant effect**
PBTB	2.0708656	0 .2032527	10.189	$< 2^{e-16}$	***
Logtb5km	-2.2286674	0.2306585	-9.662	$< 2^{e-16}$	***
BOV03	0.0002091	0.0000361	5.791	7.01^{e-09}	***
Red deer	-3.985^{e-02}	1.467^{e-02}	-2.716	0.006617	**
Roe deer	-7.058^{e-03}	2.314^{e-03}	-3.050	0.00229	**
FORkm^2	-9.887^{e-02}	2.613^{e-02}	-3.784	0.000154	***
CH0107A1	4.202^{e-02}	9.909^{e-03}	4.241	2.23^{e-05}	***
CH0107A2	-2.166^{e-02}	1.001^{e-02}	-2.164	0.030494	*
CH0114P1	-5.942^{e-03}	1.700^{e-03}	-3.496	0.000472	***
DTM	-2.204^{e-03}	7.034^{e-04}	-3.133	0.001732	**

* = P<0.05, ** = P<0.01 et *** = P<0.001; PBTB = presence of bTB the previous year; Logtb5km = logarithm of the distance to the centre of the infected pixel ; BOV03 = density of cattle in 2003; Roe deer = density per pixel; Red Deer = density per pixel; FORkm^2 = surface of the pixel occupied by forests; CH0107A1 = Land surface temperature annual amplitude (°C); CH0107A2 = Land surface temperature bi-annual amplitude (°C); CH0114P1= Normalized difference vegetation index phase of annual cycle; DTM = altitude (m)

Table II: Summary of statistics for the predictors presenting a significant relationship with the occurrence of bTB (all isolates) - Multivariate analysis – significant predictors tested together

Predictor	**Estimation**	**Standard error**	***Z* value**	***P* value**	**Significant effect**
PBTB	1.978^{e+00}	2.050^{e-01}	9,650	$< 2^{e-16}$	***
Logtb5km	-1.801^{e+00}	2.497^{e-01}	-7.213	5.48e-13	***
BOV03	2.284^{e-04}	3.855^{e-05}	5.926	3.11e-09	***
Red deer	2.205^{e-03}	5.507^{e-03}	0 .400	0.688828	
Roe deer	-1.044^{e-02}	1.988^{e-02}	-0.525	0.599500	
$FORkm^2$	-6.515^{e-02}	7.395^{e-02}	-0.881	0.378362	
CH0107A1	3.630^{e-02}	1.093^{e-02}	3.322	0.000892	***
CH0107A2	-2.135^{e-02}	1.036^{e-02}	-2.061	0.039292	*
CH0114P1	-5.094^{e-03}	1.867^{e-03}	-2.729	0.006352	**
DTM	-1.282^{e-03}	8.819^{e-04}	-1.454	0.145924	

* = P<0.05, ** = P<0.01 et *** = P<0.001; PBTB = presence of bTB the previous year; Logtb5km = logarithm of the distance to the centre of the infected pixel ; BOV03 = density of cattle in 2003; Roe deer = density per pixel; Red Deer = density per pixel; $FORkm^2$ = surface of the pixel occupied by forests; CH0107A1 = Land surface temperature annual amplitude (°C); CH0107A2 = Land surface temperature bi-annual amplitude (°C); CH0114P1 = Normalized difference vegetation index phase of annual cycle; DTM = altitude (m)

Table III: Summary of statistics for the predictors presenting a significant relationship with the occurrence of bTB (SB0162 type strains) **–** Multivariate analysis: backward selection approach

Predictor	**Estimation**	**Standard error**	***Z* value**	***P* value**	**Significant effect**
PBTB	2.0134	0.3923	5.132	2.86 $^{e-07}$	***
Logtb5km	-3.2453	0.3537	-9.176	< 2 $^{e-16}$	***
qNB	1,7932	0,6607	2,714	0,00665	**
Roe deer	-0,010778	0,004339	-2,484	0,013	*
CROPkm^2	0,08612	0,02782	3,096	0,001961	**
CH0107A1	0,053690	0,018834	2,851	0,00436	**
CH0107A2	-0,059615	0,022151	-2,691	0,00712	**
CH0114P1	-0,009562	0,003118	-3,067	0,00216	**

* = P<0.05, ** = P<0.01 et *** = P<0.001; PBTB = presence of bTB during the previous year; Logtb5kmNoI = logarithm of the distance to the centre of the infected pixel ; qNB = proportion of movements from infected pixels that took place during the current year; Roe deer density = density per pixel; CROPkm^2 = surface of the pixel occupied by crops; CH0107A1 = Land surface temperature annual amplitude (°C); CH0107A2 = Land surface temperature bi-annual amplitude (°C); CH0114P1 = Normalized difference vegetation index phase of annual cycle.

Table IV: Summary of statistics for the predictors presenting a significant relationship with the occurrence of bTB (SB0162 type strains) - Multivariate analysis: significant predictors tested together

Predictor	Estimation	Standard error	*Z* value	*P* value	Significant effect
PBTB	1.665550	0.420790	3.958	7.55^{e-05}	***
Logtb5km	-2.729520	0.381493	-7.155	8.38^{e-13}	***
qNB	1.856831	0.705736	2.631	0.00851	**
Roe deer	-0.006700	0.005178	-1.294	0.19569	
$CROPkm^2$	0.029300	0.024411	1.200	0.23003	
CH0107A1	0.038892	0.020954	1.856	0.06345	
CH0107A2	-0.059532	0.021977	-2.709	0.00675	**
CH0114P1	-0.005458	0.003630	-1.504	0.13269	

* = P<0.05, ** = P<0.01 et *** = P<0.001; PBTB = presence of bTB during the previous year; Logtb5km = logarithm of the distance to the centre of the infected pixel ; qNB = proportion of movements from infected pixels that took place during the current year; Roe deer = density per pixel; $CROPkm^2$ = surface of the pixel occupied by crops; CH0107A1 = Land surface temperature annual amplitude (°C); CH0107A2 = Land surface temperature bi-annual amplitude (°C); CH0114P1 = Normalized difference vegetation index phase of annual cycle.