Galemys, 31: 61-68, 2019
ISSN 1137-8700
e-ISSN 2254-8408
DOI: 10.7325/Galemys.2019.A7

Identification and evaluation of risk factors associated to *Mycobacterium bovis* transmission in southeast hunting areas of central Portugal

Identificación y evaluación de los factores de riesgo asociados a la transmisión de *Mycobacterium bovis* en zonas de caza del sureste del centro de Portugal

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Abstract

Some European countries have great economic Tuberculosis-related losses, so disease eradication programs have been developed, which is the case of Portugal, especially on the southeast of central region. One of the most probable cause of the eradication fail is the existence of one well-defined wildlife-cattle interface scenario and consequently a Tuberculosis (TB) cross-transmission. Understanding the field risk factors associated with TB infection in this interface is fundamental to develop effective strategies to control the bovine TB in farms. In this study, a TB risk assessment protocol was applied to 8 extensive cattle farms. 153 risk factors were identified in the study: 94 were watering points (61.4%), 40 feeding points (26.2%), 12 food storage points (7.8%) and 7 of other factors (4.6%). These points were mostly classified as high-risk factors in wildlife-cattle interface, once they enable the aggregation, cross-transmission and reveal characteristics that permit the survival of mycobacteria. Our experience suggests that it is necessary a specific design farm biosecurity plan according to the risk factors present in the field. It is needed the involvement of farmers, hunters and health authorities to assist to some progress in this fight against TB dissemination.

Keywords: cattle, game, Tuberculosis, wildlife.

Resumen

Algunos países europeos tienen grandes pérdidas económicas relacionadas con la tuberculosis, por lo que se han desarrollado programas de erradicación, como es el caso de Portugal, especialmente en el sureste de la región central del país. Una de las causas más probables del fracaso de la erradicación es la existencia de un escenario bien definido de interfaz entre la vida silvestre y el ganado y en consecuencia, una transmisión cruzada de Tuberculosis (TB). Comprender los factores de riesgo en campo asociados con la infección de TB en esta interfaz es fundamental para desarrollar estrategias efectivas para controlar la TB bovina en explotaciones ganaderas. En este estudio se aplicó un protocolo de evaluación de riesgo de TB a 8 explotaciones ganaderas extensivas. Se identificaron 153 factores de riesgo en el estudio: 94 fueron puntos de agua (61,4%), 40 puntos de alimentación (26,2%), 12 puntos de almacenamiento de alimentos (7,8%) y 7 de otros factores (4,6%). Estos puntos se clasificaron principalmente como factores de alto riesgo en la interfaz fauna-ganado, una vez que permiten la agregación, la transmisión cruzada y revelan características que permiten la supervivencia de las micobacterias. Nuestra experiencia sugiere que es necesario un plan de bioseguridad específico para la explotación ganadera de acuerdo con los factores de riesgo presentes en el campo. Se necesita la participación de los agricultores, los cazadores y las autoridades sanitarias para ayudar a avanzar en esta lucha contra la diseminación de la TB.

Palabras clave: caza, fauna salvaje, ganado, Tuberculosis.

Introduction

Animal Tuberculosis (TB) is a chronic zoonotic disease caused by mycobacteria, that belongs to the *Mycobacterium tuberculosis* complex (MTC) (Ferré 2010). The potential role of wildlife in the maintenance and spread of bovine TB in domestic livestock has been widely reported in countries all around world, especially in Europe (Gortázar *et al.* 2007). Some European countries have great economic TB-related losses. To avoid this, disease eradication programs have been developed, which is the case of Portugal. In some regions of Portugal, like the southeast of central region, difficulties in a positive progress of the program could be related with potential transmission from wildlife to cattle (Cunha *et al.* 2011).

In this portuguese affected area, extensive cattle are the main domestic TB host and have a well-defined connection with some wild TB hosts, especially when food and water are scarce resources (Vieira-Pinto *et al.* 2011). In this multi-host scenario, wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*), have a well-known role in the TB-system, but badger (*Meles meles*) and red fox (*Vulpes vulpes*) are also present in the area and play an undefined role in TB cross-transmission processes (Gortázar *et al.* 2011, Acevedo *et al.* 2014). Frequently we can observe an interaction and contact between wild and domestic species, which we call interface. This wildlife-domestic interface can comprise positive and negative effect effect on the co-existing population. Sometimes occur a bidirectional pathogen transmission between wild and domestic animals with no exception to mycobacteria (Gortázar *et al.* 2007, LaHue *et al.* 2016).

The TB pathogeny and infection are different for each host. These characteristics depends on the infection way (e.g. respiratory or alimentary way), infectious dose and host sensitivity (Corner 2006, Palmer 2013). Another point of interest is how and why the bidirectional transmission between domestic species and wildlife occurs. In the present interface a raise of bovine-wildlife interaction in risk areas is observed as a result of an alteration of the habitat and natural environment, usually due to anthropogenic causes. This change provides a modification in the structure and dynamics of wildlife populations, promoting the search for feed in different areas, especially in direction to areas where farmers supply fodder to their domestic animals (Di Marco *et al.* 2012). The transmission depends on frequency and type of domestic-wild animals contact. Recent studies in Spain prove that the indirect contact is more frequent than the direct, which is detected more in warm season and in specific sites, such as feeding and watering points. To beef cattle farms, the main risk factor to TB is effectively the presence of wildlife in their area, together with an intensive management and artificial supplementation of cattle and game species (Cowie *et al.* 2015).

Understanding the risk factors associated with TB infection in the cattle-wildlife interface and the consequent bidirectional transmission (LaHue *et al.* 2016), is fundamental to develop effective and feasible strategies scientific-based to mitigate risks of wildlife transmission and finally control TB in cattle (Di Marco *et al.* 2012, Gortázar *et al.* 2015). In this study, we aimed, for the first time in Portugal, to identify and value, in a quantitative scale, field risk factors involved in a potential TB transmission at the wildlife-livestock interface in hunting areas of southeast of central Portugal.

Material and methods

Area of study

The study area is located in Castelo Branco district, in the southeast of central Portugal, a high-risk TB area. The farms studied were in 4 parishes of Idanha-a-Nova county (Penamacor, Rosmaninhal, Segura and Zebreira) and one parish of Vila Velha de Rodão county (Fig. 1), in a total of 5 parishes in 2 counties of the TB high-risk area (DGAV 2011).

These places have unique characteristics (climate, agricultural and hunting activities) for the development and transmission of TB in a multi-host system, including cattle and wildlife species, specifically wild game, like wild boar and red deer (Cunha *et al.* 2011). These 2 counties of southeast of central Portugal, together have 132 approved hunting areas (associative, municipality and touristic) (ICNF 2017). The choice of the 8 farms studied was made according to the number of hunting areas present in each parish and the potential risk to the farms (Table 1).

Field protocol

The protocol used in this study was presented in recent international scientific meetings. We applied the same TB risk assessment protocol to 8 cattle farms in southeast of central Portugal. This protocol includes the following steps (Martínez-Guijosa *et al.* 2016):

- Contact and structured-interview with the farmers (general information about farm characteristics and management, categorization of the wildlife species present in the farm): farm area, stock rate, production type, land use, feeding and water management, wildlife presence in farm

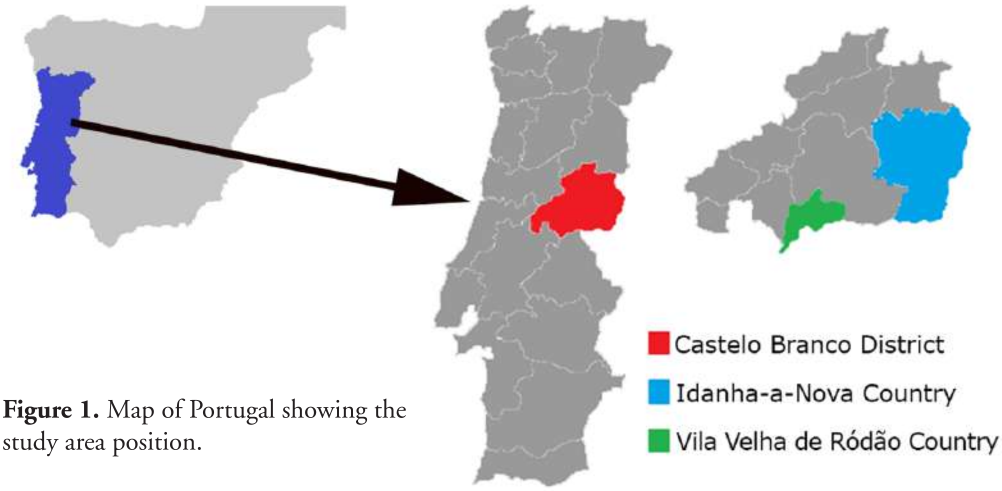

Figure 1. Map of Portugal showing the study area position.

Table 1. General characteristics of the 8 study farms.

Farm	Management	Size (ha)	Stock rate (cattle/ha)	Presence of another domestic specie	Presence of wild species
A	Cattle	800	0.33	Goat and sheep	Fallow deer, Badger and fox (+++) Wild boar and red deer (++)
B	Cattle	250	0.35	Sheep, horse and poultry	Wild boar, red deer and fox (++)
C	Cattle + Game	120	0.38	Poultry	Wild boar (++) Red deer and fox (+)
D	Cattle + Game	110	0.41	Poultry	Wild boar (++) Red deer and fox (+)
E	Cattle + Game	1,000	0.23	Horse	Wild boar and red deer (+++) Fox (++)
F	Cattle + Game	300	0.12	---	Wild boar, red deer, fox and badger (+++)
G	Cattle + Game	54	0.72	---	Fox (+)
H	Cattle + Game	800	0.09	Sheep and poultry	Wild boar, red deer, fox and badger (+++)

and quantification of this presence, type of game in the farm (large or small game) and artificial supplementation of water and feed to cattle and game.

- Farm limits (perimetral and internal) mapping with Google Earth® and *BatchGeo*®.
- GPS location, photography and characterizing in field of TB risk points.
- Reporting the main information with recommended measures that must be implemented to reduce the risk for interspecific contacts.

Data analysis

In this study, the 153 risk factors analysed in the 8 cattle farms (fitted in 4 categories: 94 watering points, 40 feeding points, 12 feed storage points and 7 other factors) were classified in a risk scale (1-minimum and 5-maximum risk), according to the characteristics presented in field and to the protocol designed by the Institute of Game and Wildlife Research (IREC) (Martinez-Guijosa *et al.* 2016).

The watering risk points were classified considering the following characteristics: pond diameter, water troughs' height, existence of enclosures, wildlife access and imprints *in loco,* drying period, sun exposure and local cleaning. To the feeding points, was taken into account: quantity and frequency of feed administration, feeder's height, wildlife access and presence of feed waste and dirtiness. The feed storage points were classified according to the type of closure, feed storage conditions and humidity and dirtiness presence. The other factors were specific of the farms, with us taking into account essentially the structure, cleaning and wildlife presence.

Additionally, in order to complete the report, it was necessary to include information on health status of the livestock during the previous 5 years (2012-2017), based on official data; and cohabitant large game species health status, which information was obtained through hunted animals' sanitary inspection data *in loco* (hunting seasons 2012/2013 to 2016/2017).

Table 2. Watering risk factors by farm and respective risk classification.

Risks factors		Risk	Farms								Total
			A	B	C	D	E	F	G	H	
Watering points	Ponds	0									-
		1									-
		2	1		1	1			3		6
		3	3		4		2		3	1	13
		4	6	1	1	1	15	5	4	1	34
		5	2	3	2	1	1	1	2		12
	Streams	0									-
		1									-
		2	3			1	1				5
		3				1				1	2
		4	2					4			6
		5							1		1
	Water troughs	0				1			4		5
		1									-
		2			1				1		2
		3		1	2	2			1		6
		4									-
		5								2	2
Total			17	5	11	8	19	10	19	5	94

Results and discussion

A total of 153 risk factors were identified in the field of the 8 farms with 94 of them coming from watering points (61.4%), 40 from feeding points (26.2%), 12 from feed storage points (7.8%) and 7 of them from other factors, like husbandry support structures (4.6%). Generally, our results support the previous related literature.

The results on water points pointed out to the main relevance of these resources as a TB risk factor for cattle (Table 2). It is important to identify the water management in the farm to perform a proper evaluation of the risk associated to TB transmission in these points (Martínez-López *et al.* 2014). The orography of the study area allows the use of natural water points, such as ponds. The fact that the ponds had the largest number of risk factors (69.1%) found in the class of watering points deserves to be highlighted. Our results confirm the high risk associated to natural ponds, that is described in previous studies in the Mediterranean area, once the characteristics of these natural points are favourable to the *M. bovis* survival and in dry season it is a great aggregation area (Kukielka *et al.* 2013, Cowie *et al.* 2015, Santos *et al.* 2015). In addition, our study area is part of the Mediterranean climate zone where most of the farms, and especially the natural

Table 3. Feeding risk factors by farm and respective risk classification.

Risks Factors		Risk	Farms								Total
			A	B	C	D	E	F	G	H	
Feeding points	Feeders	0			1						1
		1									-
		2			1						1
		3	1		2						3
		4	2	2	1		7	7	4	2	25
		5									-
	Food in soil points	0									-
		1									-
		2							1		1
		3									-
		4		1			1	1			3
		5								1	1
	Sowings	0									-
		1									-
		2									-
		3									-
		4								1	1
		5					1				1
	Feeders for large game	0									-
		1									-
		2									-
		3									-
		4									-
		5								3	3
Total			3	3	5	-	9	8	5	7	40

water points are localized in shady areas and forests and consequently it is provided the ideal humidity, light and temperature conditions to the survival of *M. bovis* (Humblet *et al.* 2009).

The artificial watering points, like cattle water troughs, have some importance in this TB research. When the natural water is scarce, wildlife look for other water sources. Due to this fact, these water troughs became not exclusive for the cattle's use. Consequentially, they are considered aggregation points in interface areas (Martínez-López *et al.* 2014).

The feed risk points analysed in our study represent secondary major class of risk factors (Table 3) (Ferré 2010, Nugent *et al.* 2015). Regarding the feeding points, it is important to know where and when the cattle feed is provided. Where? If there were feeders or the feed was dispensed in the soil. When? Once or twice a day and what time of the day. Another related problem is the existence (or not) of supplementary feeding to wildlife for game purposes.

Non-existence of selective feeders in cattle farms is dangerous to healthy animals, once literature refers that 18% of samples of soil near of feeders contain *M. bovis* with infectious capacity, and non-selective feeders permits cohabitation and contact between cattle and wildlife (Santos *et al.* 2015). Cross-transmission is more evident in dry season, when natural feed is scarce in the environment (Vicente *et al.* 2007).

The time of the day when farms provide extra feed to cattle can be a relevant risk factor in the farm's management. Farmers who provide feed in the late afternoon have the risk of cohabitation and cross-transmission increased, because it is the main hour when game species search for new feed in the environment (Martínez-López *et al.* 2014). The artificial feed supplementation is identified as the main risk factor to transmission, once they attract game species (Vicente *et al.* 2013). It was proved that wild boar aggregation in artificial feeders result in an increased number of TB infected wild boars and has a positive correlation with the increase of positive TB red deer (Vicente *et al.* 2006).

To provide feed supplementation to cattle and game species it is necessary a farm storage. In the storage, the hay and concentrate feed must be out of range of cattle, wild boar, red deer and another wildlife species, like badgers and little rodents (Santos *et al.* 2009). In England, for example, the main TB wildlife host is the badger, and they cause a huge economic problem with the TB transmission to dairy cattle (Gortázar *et al.* 2011). The importance of a good feed storage comes from of the evidence that *M. bovis* survive in the hay till 55 days in good conditions (Santos *et al.* 2015). In our study, only one farm had a storage with risk level 2, once it presented a semi-open storage with railings that allowed the access of smaller mammals, like rodents and badgers. The other ones had closed storages with good food protection conditions.

Conclusions

The watering and feeding points are potential aggregation points of bovine and large game which benefit the transmission in the wildlife-cattle interface. In this study, these points were mostly classified as high-risk factors, once they facilitate the aggregation and reveal characteristics that permit the survival of mycobacteria. Our experience suggests that it is needed the designing of farm specific biosecurity plans according to the field's specific risk factors, since a standard design does not work similarly in all the farms/hunting areas where TB risk is evident. It is needed the involvement of farmers, hunters and health authorities to assist to some progress in this fight against TB infection on game and farm animals.

Acknowledgements

This study was funded by the project UID/CVT/00772/2013 and UID/CVT/00772/2016 supported by the Portuguese Science and Technology Foundation (FCT). Pelayo Acevedo is supported by MINECO-UCLM through a "Ramón y Cajal" contract (RYC-2012-11970) and partly by the project AGL2016-76358-R. Jordi Martinez-Guijosa is supported by the MINECO FPI pre-doctoral scholarship (BES-2015- 072206). The authors would like to thank to Dr. António Manteigas, from Veterinary Services of Castelo-Branco-DIVCB and all farmers and hunting managers, for all the help provided during the development of this study.

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Submitted: 14 May 2019
Accepted: 22 October 2019

Associate editor was L. Javier Palomo