



# ERCIYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

## Journal of Faculty of Veterinary Medicine, Erciyes University

Research Article / Araştırma Makalesi  
20(3), 156-161, 2023  
DOI: 10.32707/ercivet.1387764

### Analysis of General and Disease-Specific Risk Variables for Bovine Tuberculosis

Berrin ŞENTÜRK<sup>1,a</sup>, Aytaç AKÇAY<sup>2,b</sup>, Savaş SARIOZKAN<sup>3,c</sup>, Mehmet KÜÇÜKOFLAZ<sup>4,d</sup>

<sup>1</sup>Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Animal Health Economics and Management, Samsun-TÜRKİYE

<sup>2</sup>Ankara University, Faculty of Veterinary Medicine, Department of Biostatistics, Ankara-TÜRKİYE

<sup>3</sup>Erciyes University, Faculty of Veterinary Medicine, Department of Animal Health Economics and Management, Kayseri-TÜRKİYE

<sup>4</sup>Kafkas University, Faculty of Veterinary Medicine, Department of Animal Health Economics and Management, Kars-TÜRKİYE

ORCID: <sup>a</sup>0000-0002-0455-9341; <sup>b</sup>0000-0001-6263-5181; <sup>c</sup>0000-0003-2491-5152; <sup>d</sup>0000-0003-3256-4735

**Corresponding author:** Savaş SARIOZKAN; E-mail: ssariozkan@erciyes.edu.tr

**How to cite:** Şentürk B, Akçay A, Sariozkan S, Küçükoflaz M. Analysis of general and disease-specific risk variables for bovine tuberculosis. Erciyes Univ Vet FakDerg 2023; 20(3): 156-161

**Abstract:** In this study, the relationship of risk variables to the incidence of bovine tuberculosis in areas where the disease is commonly seen and rarely seen was investigated in the years of 2017. For this purpose, the districts of Samsun Province in Türkiye were divided into three risk groups according to the number of bovine tuberculosis outbreaks. The disease-specific risk variables were surveyed 300 cattle enterprises. Information was collected on the feeding regime of the animals, access of drinking water, the source of feed, the management of the manure waste, the presence of wild animals in the area and the presence of similar enterprises within 150 meters. In the analysis of the risk variables associated with the disease, 11 variables were evaluated and a total risk score was allocated. The relationship between the number of outbreaks and general risk scores was significant ( $P<0.05$ ). Moreover, a significant difference was determined between the districts in terms of disease-specific risk variables ( $P<0.05$ ). In conclusion, the results of this study can assist in the determination of the vulnerability of individual farms and particular areas to bovine tuberculosis outbreaks, reduce the risk of disease outbreak, and also facilitate early intervention and suppression of the disease.

**Keywords:** Bovine tuberculosis, disease management, disease specific risks, scoring, Türkiye

#### Sığır Tüberkülozu için Genel ve Hastalığa Özgü Risk Değişkenlerinin Analizi

**Öz:** Bu çalışmada, 2017 yılında hastalığın sık görüldüğü ve nadir görüldüğü bölgelerde risk değişkenlerinin sığır tüberkülozu insidansı ile ilişkisi araştırılmıştır. Bu amaçla Türkiye'de Samsun iline bağlı ilçeler sığır tüberkülozu salgınlarının sayısına göre üç risk grubuna ayrılmıştır. Hastalığa özgü risk değişkenleri 300 büyük baş hayvan işletmesinde incelenmiştir. Hayvanların beslenme rejimi, içme suyuna erişimi, yem kaynağı, gübre atıklarının yönetimi, alanda yabani hayvanların varlığı ve 150 metre yakınındaki benzer işletmelerin varlığı hakkında bilgi toplandı. Hastalıkla ilişkili risk değişkenlerinin analizinde 11 değişken değerlendirildi ve toplam bir risk puanı belirlendi. Salgın sayısı ile genel risk skorları arasındaki ilişki anlamlı bulundu ( $P<0.05$ ). Ayrıca ilçeler arasında hastalığa özgü risk değişkenleri açısından da anlamlı bir fark saptandı ( $P<0.05$ ). Sonuç olarak, bu çalışmanın sonuçları, bireysel işletmelerin ve belirli alanların sığır tüberkülozu salgınlarına karşı savunmasızlığının belirlenmesine yardımcı olabilir, hastalık salgını riskini azaltabilir ve ayrıca hastalığın erken müdahalesini ve baskılanmasını kolaylaştırabilir.

**Anahtar kelimeler:** Hastalık yönetimi, hastalığa özgü riskler, sığır tüberkülozu, skrolama, Türkiye

#### Introduction

Epidemic diseases have important negative effects on people, animals and the global economy. Currently, countries use different types of cattle tuberculosis (bTB) fighting methods according to their economic development levels. Among these methods of struggle, risk-focused disease control management is increasing its importance. In the case of bTB infection, the ingestion of the active substance into a new host

usually takes place through the respiratory or digestive tract. In the case of animals in barns, the respiratory tract is the primary route of infection, while in pastures the routes are the respiratory and digestive tracts via infected food and water sources. On farms, most young animals are infected after ingesting contaminated food (Radostits et al., 1994). In addition, the infection can be caused by badger or ferret bite (Kaneene and Pfeiffer, 2006). It has also been reported that the disease can be carried on the feet of birds and in their faeces (Witmer et al., 2010). The Kızılırmak Delta and its Bird Sanctuary, located on

Geliş Tarihi/Submission Date : 09.12.2022  
Kabul Tarihi/Accepted Date : 08.03.2023

the borders of the Bafra district (Samsun province of Türkiye), should paid more attention when biosecurity measures are implemented, especially in the nearby settlements. In addition, there are many other studies reporting the relevance of wildlife to the occurrence of the disease (Aranaz et al., 2004; Atılğan et al., 2006; Fitzgerald and Kaneene, 2013; Little et al., 1982; Orton et al., 2018; Renwick et al., 2007). In the event of a disease outbreak on a specific farm, other farms in its vicinity are at risk. The distance between farms should be determined, especially in terms of the distance between the nearest manure piles, when investigating the potential for the spread of the disease. In order to minimize the potential for manure to be a source of the disease, there should be a minimum distance of 150 m to the nearest farm for small-scale cattle farms (Atılğan et al., 2006). However, for cattle breeding enterprises, the distance to the nearest farm in about 90% of cases was less than 150 meters in Turkey (Varol and Atılğan, 2017). The potential contamination of water resources requires consideration of the factor. In the case of large farm herds, for example 600 head of mixed stock or 430 head of dairy cattle, there should be a minimum separation distance of 450 m (Liang and Van Devender, 2010). Another important source of risk is the distance from the livestock to the water source for the enterprise. This distance is generally 40 m or less and it influences the risk presented by manure and water contamination in livestock enterprises in the Mediterranean Region (Atılğan et al., 2006).

Bovine tuberculosis is a zoonotic disease, caused by the bacterium, *Mycobacterium bovis* (Milan-Suazo et al., 2010; Evans et al., 2007; Sunder et al., 2009). This disease was selected as a research topic because of its relevance to Samsun Province, Turkey, and also because of its zoonotic character and the variety of routes of transmission.

In this study, the applicability of the risk-based approach to the epidemic diseases has been investigated. The studies to be carried out for this purpose will enable the development of strategies and practices that minimize the risk of outbreaks in the areas where epidemics are seen, as well as the implementation of preventive measures to protect low risk areas.

In the light of this information, the aim of this study was to investigate the risk variables applicable to outbreaks of bTB in Samsun Province, Türkiye because they harm both human and animal health and cause substantial financial losses.

## Material and Methods

The material for this study consisted of data on the risk variables relevant to bTB (Aranaz et al., 2004; Fitzgerald and Kaneene, 2013; Orton et al., 2018; Renwick et al., 2007; Sümbüloğlu and Sümbüloğlu,

2005). For this purpose, 11 general and 11 disease-specific risk variables were used. The general risk variables such as; disease statistics of the study area, livestock statistics, number of cattle by district, number of animals transported, number of slaughterhouses, demographic statistics, pasture status and number of animal health workers in the study area were used. As for disease-specific risk variables the recurrence of bTB in certain areas, wildlife and environmental risks were determined.

Detailed information on the disease was obtained from the Turkish Ministry of Agriculture and Forestry, with the permission dated 25.01.2017 and numbered 78255852-192904, and the data is defined as Official Data (OD). By using the annual activity reports of the Ministry of Agriculture and Forestry, this study was able to include data related to 11 variables, which were included as general risk variables. NCSS 2009 (Version 9.0.5, Perpetual Single-User License) package program was used for statistical analysis.

The survey, firstly, by using the outbreak data for 2010-2016 for the Bafra district, which had the highest number of outbreaks of bTB, that district was classed as being at "high risk", Havza district was termed "medium risk" and Ayvacık district, where the disease is rare was defined as "low risk". To conduct a robust sampling regime, five villages were selected from each district and a total of 300 cattle breeding enterprises were surveyed with the random sampling method (There are 1252 livestock farm in the study area). The population of this study consisted of a total of 1252 cattle farms located in Samsun province, Ayvacık, Bafra, Havza counties. In the study, the sample size was calculated as at least 295 enterprises in total and the study was carried out with 300 farms, using the 95% confidence interval and 0.05 marginal deviation in the enterprises. The calculation of the sample size ( $n_0$ ) in the study is given in the equation below.

$$n_0 = \frac{N t^2 p q}{d^2 (N - 1) + t^2 p q} = \frac{1252 (1,96)^2 0,5 * 0,5}{0,05^2 (1292 - 1) + (1,96)^2 * 0,5 * 0,5} \cong 295$$

N = Population size; t-table value for t = 95% confidence interval = 1.96; p, q = Frequency of occurrence of the event in question, from being a factor (+) and being a factor (-), p = 0.5, q = 0.5; d = marginal deviation (Sümbüloğlu and Sümbüloğlu, 2005). In the study area, a survey was conducted with approximately 100 enterprises in all three districts. Prevalence of bTB was also considered in calculating the minimum sample size.

The study was carried out in the Ayvacık, Bafra and Havza districts of Samsun Province, namely Şenpınar, Söğüt pınar, Örencik, Eynel, Koçyurdu, Doğanca, Altınay, Yakıntaş, Osmanbeyli, Kale, Kamlık, Cevizlik and Çamyatağı villages Karpuzlu and

Havza Center surroundings.

In the analysis of general risk variables, the total general risk score (Y) was calculated with the following formula (Liang and Van Devender, 2010; Radostits et al., 1994).

$$(Y) = f(a_1 + a_2 + a_3 + a_4 + a_5 + a_6 + a_7 + a_8 + a_9 + a_{10} + a_{11})$$

Where:

Y = General risks of bTB occurrence

a<sub>1</sub> = Number of study area outbreaks

a<sub>2</sub> = Proportion of total animals in the province

a<sub>3</sub> = Number of farms and average number of cattle per farm

a<sub>4</sub> = Animal product facilities at the district level (dairy farm, milk factory, livestock cooperative)

a<sub>5</sub> = Slaughterhouses by district and animal bazaar

a<sub>6</sub> = Number of people per km<sup>2</sup> in district

a<sub>7</sub> = Number of animal transports by district

a<sub>8</sub> = Districts' coastal roads, ring roads, roads connecting with other provinces

a<sub>9</sub> = The share of the province pasture by the districts

a<sub>10</sub> = Number of veterinarians and veterinary technicians working in the district (official)

a<sub>11</sub> = Number of artificial inseminations at the district level

Values from 1 - 25, 1 (lowest),

Values from 26 - 50, 2 (low),

Values from 51 - 75, 3 (medium),

Values from 76 - 100, 4 (high).

Data of the disease-specific risk variables were obtained through questionnaires. In the determination of risk variables, the risks specific to the disease, which were demonstrated in previous studies (Orton et al., 2018), were used. The data obtained from the questionnaires, which identified 11 risk variables specific for bTB and the number of animals at risk of the disease on the individual farms, were recorded electronically and a data index was created. Missing and incorrect information was identified during data entry which meant that the number of incorrect entries was minimized. Univariate statistical analysis was used to evaluate the data obtained from the surveys. The relationship between animal numbers and disease risk was also investigated in the study. Descriptive statistics (percentage distributions, mean ± standard error of mean) were performed on the 300 samples and cross-tables showing the relationships between districts and risk variables, classified according to risk levels. The Pearson Chi-square and Fisher's exact test was performed according to the scale type and number of the variables. Data were analyzed with NCSS statistical software with P values <0.05 considered significant.

### Results

The risk scores for high, medium and low risk areas for bTB, determined by using the general risk variables (a<sub>1</sub> - a<sub>11</sub>), are presented in Table 1.

**Table 1.** General risk scores for bTB across three districts in Samsun Province, Türkiye

Districts/ Region	Risk Variables											Total Risk Scores
	a <sub>1</sub>	a <sub>2</sub>	a <sub>3</sub>	a <sub>4</sub>	a <sub>5</sub>	a <sub>6</sub>	a <sub>7</sub>	a <sub>8</sub>	a <sub>9</sub>	a <sub>10</sub>	a <sub>11</sub>	
<b>Ayvacık</b>	1	1	1	-	1	1	1	-	-	1	-	7
<b>Havza</b>	2	2	2	2	2	1	2	2	2	2	2	21
<b>Bafra</b>	3	3	2	3	2	3	3	3	3	2	3	30

The lowest value for the risk scoring system was 1 and the highest was 4. The total risk score was obtained from the sum of the risk scores for each risk variable.

Ordinal scoring (1 through 4) was made according to the risk status. Hierarchical clustering analysis method was used to determine the number of risk groups and according to the total score values. In scoring, the sum of the data scores for all three districts was taken to be 100 points and the proportion of the districts from the total score was divided into 4 equal parts.

The data and its statistical evaluations for different risk levels of the disease are presented in Tables 2 and 3.

The number of bTB outbreaks across the three districts, specific risk variables, there were significant differences (P<0.05) for the type of feeding of animals, drinking water source of animals, the management of waste, application of biosecurity measures, farm close to wildlife habitat, the presence of goats and/or sheep in the common area, and the presence of animals on a different farm within 150 meters.

There were significant differences in the mean number of animals per farm in the districts categorized according to the number of tuberculosis outbreaks (P<0.05).

**Table 2.** Distribution of disease-specific bTB risk factors for cattle by district in Samsun Province, Türkiye

Risk factors	Districts			P
	Low Risk Ayvacic (%)	Medium Risk Havza (%)	High Risk Bafra (%)	
<b>1. Feeding areas of animals</b>				
Exclusive use of external areas	99(99.0)	12(12.0)	45(45.0)	<0.001
Non-use of external areas	1(1.0)	88(88.0)	55(55.0)	
<b>2. Drinking water sources</b>				
Tap water	1(1.0)	21(21.0)	40(40.0)	<0.001
Well or spring water	99(99.0)	2(2.0)	9(9.0)	
Common (fountain-stream)	0(0.0)	77(77.0)	51(51.0)	
<b>3. Manure management on farm</b>				
Accumulating near barn on farm	74(74.0)	91(91.0)	89(89.0)	0.001
Removed to field	26(26.0)	9(9.0)	11(11.0)	
<b>4. Biosecurity (e.g. ventilation, environmental control, staff hygiene)</b>				
Applied	23(23.2)	17(17.0)	23(23.0)	0.476
Not applied	76(76.8)	83(83.0)	77(77.0)	
<b>5. Additional biosecurity measures taken after the outbreak of epidemic disease</b>				
Yes	43(43.0)	83(83.0)	92(92.0)	<0.001
No	57(57.0)	17(17.0)	8(8.0)	
<b>6. Badger - seen in the potential contact area in the previous 12 months</b>				
Yes	18(18.0)	39(39.0)	35(35.4)	0.002
No	82(82.0)	61(100.0)	64(64.6)	
<b>7. Pig - seen in the potential contact area in the previous 12 months</b>				
Yes	68(68.7)	66(66.0)	24(24.0)	<0.001
No	31(31.3)	34(34.0)	76(76.0)	
<b>8. Other possible carrier wild animals seen in the potential contact area in the last 12 months</b>				
Yes	57(57.0)	30(30.0)	15(15.0)	<0.001
No	43(43.0)	70(70.0)	85(85.0)	
<b>9. Goats present in the common area</b>				
Yes	0(0.0)	49(49.0)	2(2.0)	<0.001
No	100(100.0)	51(51.0)	98(98.0)	
<b>10. Sheep present in the common area</b>				
Yes	0(0.0)	55(55.0)	36(36.0)	<0.001
No	100(100.0)	45(45.0)	64(64.0)	
<b>11. Farm with animals within 150 meters</b>				
Yes	63(63.0)	88(88.0)	85(85.0)	<0.001
No	37(37.0)	12(12.0)	15(15.0)	

**Table 3.** Number of animals in districts classified by level of risk (Mean ± SE)

Variables	Districts			P
	Ayvacic	Havza	Bafra	
Number of animals on farms	6.51±0.50 <sup>a</sup>	11.29±1.35 <sup>b</sup>	31.4±4.07 <sup>c</sup>	<0.001
Number of female cattle	3.94±0.24 <sup>a</sup>	7.44±0.60 <sup>b</sup>	21.44±2.7 <sup>c</sup>	<0.001
Number of male cattle	2.57±0.42 <sup>a</sup>	3.87±1.20 <sup>b</sup>	9.96±1.74 <sup>c</sup>	<0.001
Number of young animals (< 3yrs)	3.16±0.37 <sup>a</sup>	5.28±1.20 <sup>b</sup>	13.42±1.90 <sup>c</sup>	<0.001
Number of adult animals (3-8 yrs)	2.56±0.22 <sup>a</sup>	4.51±0.39 <sup>b</sup>	14.18±2.10 <sup>c</sup>	<0.001
Number of animals (> 8 yrs)	0.79±0.10 <sup>a</sup>	1.49±0.21 <sup>b</sup>	3.75±0.68 <sup>c</sup>	<0.001

<sup>a,b,c</sup>: The difference between means in the same row with different letters is significant.

## Discussion and Conclusion

In areas where bovine tuberculosis has historically been detected, the risk of recurrence is greater than in areas where the disease has not been seen (Orton et al., 2018). In this research, it was argued that in the areas where the disease risk is high, explaining this situation only by animal movements is not sufficient. It was further argued that local factors contribute to the spread of the disease. Determining the high-risk areas before outbreaks of the disease is the key to minimizing the level of risk associated with different contributing factors (Orton et al., 2018). These explanations are relevant to the Bafra district, which has a high number of outbreaks and was defined as high risk; it is therefore necessary to develop specific measures that take account of the historic frequency of the disease. In the Bafra area, the need for drinking water was mostly met from the common grazing area (51%), there was a high rate of disposal of farm waste due to animal density (89%) and the number of badger sightings (35%) was higher than in other districts. This indicates the need to develop area specific measures for the prevention and control of the disease.

In this study, in the analysis of general risk variables using the 1- 4 scoring method for the disease, the total risk score of the Bafra district, where the disease risk was determined to be the highest, was 30, in the medium risk Havza district was 21, and in the low risk Ayvacık district was 7. The general risk scores in this study were similar to those reported for the relationship between global risk factors and the number of outbreaks for the Foot and Mouth disease, in terms of general risk variables (Şentürk et al., 2016). This situation suggests that the methods used in the study may be applicable to the determination of the general risk variables characteristic for epidemics.

The on-farm disposal of manure should be at least 150 m from the nearest neighbouring farm (Atılğan et al., 2006) but on 89.5% of the cattle farms in Turkey the waste disposal site was less than 150 m from the closest neighbour (Varol and Atılğan, 2017). In the present study, in the Bafra district, which was in the high risk category, the manure deposition site on 85% of farms was less than 150 m from the nearest neighbour. The results of this study have again demonstrated that cattle farms in close proximity are at higher risk of the spread of bTB from their neighbours. The close proximity of farms to each other therefore constitutes a significant risk for the transmission of the disease. The highest rate of use of the external/outside areas of the farms was in Ayvacık (99%) and the difference between it and the other districts was significant ( $P < 0.001$ ). This suggests that increasing the density of grazing animals on common pasture areas increases the risk of exposure to the disease and hence transmission of the disease.

It is not appropriate to feed animals susceptible to disease in the areas where they are at risk of exposure to animals that are thought to be carriers of the disease (Fine et al., 2011). This potential threat was investigated in the present study and in Bafra (high risk) and Havza (medium risk) the cattle were being exposed to sheep on the same pastures. It is therefore imperative that measures be taken to increase the awareness of owners to prevent the interaction of different species in the common grazing areas. In this study, the inadequacy of bio security practices was also demonstrated in all three districts, which were at different risk levels. Considering the survival time of the disease pathogen in different environments, minimizing both the direct (common pastures) and indirect (manure piles) exposure of animals of one farm to sources of infection from other farms should always be emphasized as high priorities in educational programs.

Separately, significant differences were found between the average number of animals, males, females, young animals, adult animals and old animals in the three districts and the number of bTB outbreaks ( $P < 0.05$ ). These results imply higher rates of disease associated with higher numbers of animals in the high-risk areas which would result in higher economic losses due to the disease.

Risk assessments in disease control are essential in developing countries where financial resources for disease control are scarce (Avila et al., 2018). On the other hand, in the fight against disease local spread will require a deeper understanding of the patterns, drivers and characteristics (Milne et al., 2022). The study supported the contention that the general disease risk factors do not change much in epidemics in different situations and with different characteristics. Consequently, practical disease prevention measures should be implemented urgently to reduce the disease risk across all areas, especially in the high risk areas. The results of this study, which was conducted with a view to the development of improved disease prevention and control strategies, strongly suggest that the following measures be taken:

-In the areas where the disease arises and the common pasture area is used, animals should be used tubercle test.

-Temporary prohibition of the use of these pastures (taking into account the life span of disease agent in the soil and water)

-On farms where the disease is detected, even if the farm is later declared free from the disease, animals should not be sold for breeding or fattening purposes for a mandatory minimum period but these farms should be able to sell for slaughter. With respect to these three steps, financial support should be provided.

ed to prevent the producer from being disadvantaged. When a new farm is established, the distance to the nearest farm should be more than 150 m so as to minimize the potential for the transmission of the disease,

-Disease prevention and control measures should take into account the number of previous infections on a particular farm,

- Investigate the presence of the disease in wildlife, especially the badger, which is reported to be a disease carrier.

In conclusion, this study determined that a set of eleven general and eleven disease-specific risk variables can be used to classify the risk an outbreak of bTB in a particular area. Risk-based control strategies should be adapted to the system in order to increase efficiency in disease intervention and control.

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