Prevalence of bovine tuberculosis in cattle in Alborz Province, Iran between 2011 and 2021

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Abstract

Bovine Tuberculosis (bTB) is a zoonotic infection with global distribution and is considered a major public health concern particularly in low- and middle-income countries such as Iran. The objective of this cross-sectional study was to determine the screening coverage, individual prevalence, and the number of slaughterhouse condemnations in the cattle in Alborz province, Iran between 2011 and 2021.The epidemiological data of bTB were retrieved from the Geographic Information System of Veterinary Organization of Alborz, Iran. The Prevalence rates were estimated for cattle and pregnant cattle. For temporal analysis, the prevalence of tuberculin-positive cattle were charted according to year. For spatial analysis, the overall prevalence of tuberculin positive and suspected cattle was mapped. The highest and lowest coverage of herds were in Nazarabad (n=1379) and Taleqan (n=54), respectively. The overall prevalence was 0.05% with the peak of 0.11% in 2013 and an increasing trend between 2018 and 2021 was observed. The overall average age of tuberculin-positive cattle was estimated to be 3.55±0.75 years. The mean size of reaction to tuberculin inoculation was 7.21±2.70 mm. The highest overall individual prevalence of tuberculin positive and suspected cases was observed in Taleqan (0.1243%) and Chahar Bagh (0.0751%). The relative frequency of pregnancy was 31.45% displaying an increasing trend. The highest frequency of lesions in the slaughterhouse condemnations belonged to mesenteric lymph node lesions (n=206) and pulmonary and laryngeal lesions (n=92).Based on our findings, bTB is present in all cities of Alborz and more thorough investigation are suggested.

Keywords: Bovine Tuberculosis, Mycobacterium bovis, Cattle, Iran

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Introduction

Bovine Tuberculosis (bTB) is a zoonotic infection with global distribution (Barnes *et al.*, 2023). The causative agent of bTB is primarily *Mycobacterium bovis*. However, other members of the Mycobacterium tuberculosis complex such as *M. tuberculosis*, *M. africanum*, *M. caprae*, *M. orygis*, and *M. microti* are also able to cause bTB in cows (Ramanujam *et al*., 2023). Although M. bovis is able to infect a wide range of mammalian species, cattle are considered to be the primary host of bTB (Klepp *et al*., 2023), which causes significant economic losses due to the condemnation of the infected organs and carcasses of the slaughtered cattle, losses in the dairy industry due to decreased productivity, culling, and reduced the rate of calving, and trade restrictions (Kapalamula *et al*., 2023; Zhu *et al*., 2023). In ruminants, the intradermal tuberculin test (Toribio *et al*., 2023) is suggested by the World Organization for Animal Health via measuring the diameter of the delayed hypersensitivity reaction caused by tuberculin (Lin *et al*., 2022). However, bacterial culture and molecular methods such as Polymerase Chain Reaction (PCR) have also been validated to be utilized as diagnostic tests in animal tissues (Morris *et al*., 2023).

Due to the zoonotic nature of M. bovis, it is considered a public health concern, especially in low- and middle-income countries (Devi *et al*., 2021) including Iran (Mahdaviani *et al*., 2020). It has been estimated that 12.1% of all cases of human tuberculosis are caused by M. bovis worldwide (Taye *et al*., 2021). Despite human tuberculosis been mostly caused by M. tuberculosis, M. bovis still plays an important role in human tuberculosis infections, particularly in developing countries due to its significant prevalence in the livestock (Rodríguez-Hernández *et al*., 2023). Livestock farmers, abattoir workers, animal husbandry workers, HIV-positive individuals, the personnel working in veterinary sectors, butchers, hunters, wildlife workers and live market workers are sub-populations at higher risk of infection with M. bovis (Devi *et al*., 2021). Moreover, bTB causes significant economic losses including pruning production, the costs of screening, culling the infected animals, and trade restrictions (Mohamed., 2019). This study aimed to estimate the screening coverage, individual prevalence, and the number of slaughterhouse condemnations in cattle between 2011 and 2021 in Alborz province, Iran.

Materials and methods

The data of number of cows, number of farms, year, age, city, type of farm (industrial/traditional in dairy/beef farms), history of pregnancy, history of tuberculin test of the herd (number of positive cases and relative frequency), slaughter house condemnations based on the clinical forms and type of legions of suspected and definitive diagnosis of bTB in industrial and traditional cattle farms between 2011 and 2021 in all cities of Alborz province were obtained from the Geographic Information

System (GIS) of Veterinary Organization of Alborz, Iran. The age was categorized to less than 2, 2-3, 3-4, 4-5, and more than 5 years for tuberculin-positive cattle. Multivariate linear regression was used to determine the effects of age categories and year on the total number of positive cases.

The prevalence rate of bTB was estimated for cattle based on the number of tuberculin-positive and the total number of cattle that were tested in Alborz province based for each year and overall for the time period of 2011 and 2021. Data were summarized with descriptive statistics. All statistical analysis was performed with SPSS: 26. For temporal analysis, individual prevalence and relative frequency of pregnancy were charted based on year. For spatial analysis, a map for 2011-2021 period was drawn for the cities. And, frequency of tuberculin positive and suspected cases were displayed per 100000 individuals. Spatial analysis was carried out with QGIS 3.34.1.

Results and discussion

Study population characteristics

Between 2011 and 2021, overall, 1698211 cattle from 3263 farms were tested by tuberculin test. Based on year, the highest number of tested farms was observed in 2014 and 2015, when 379 and 376 farms were tested, respectively. And, the lowest number of tested farms was in 2011, 2012, and 2013, when 202 farms were tested each year.

Seven cities were investigated in this survey including Eshtehard, Chahar Bagh, Savojbolagh, Taleqan, Fardis, Karaj, and Nazarabad. The coverage of tuberculin screening was not similar among the cities with the highest number of tested farms belonging to Nazarabad (n=1379) and Savojbolagh (n=852) and the lowest number of tested farms belonging to Eshtehard (n=72) Taleqan (n=54) (Table 1). The highest coverage belonged to industrial (n=1781) and traditional (n=855) dairy farms whilst the coverage for industrial and traditional beef farms were 35 and 7, respectively.

Total number of positive cases

In multivariate linear regression, the coefficients of age categories and year was not statistically different from zero ($p<0.05$), suggesting that no linear relation is present between the average number of positive cases and year and age categories of the positive cattle.

Individual prevalence

Between 2011 and 2021, from 1698211 tested cattle, 849 were found to be positive by tuberculin test. And, the overall prevalence was 0.05% with the peak of 0.116% in 2013 (Table 2). However, fluctuation with an increasing trend between 2018 and 2021 was observed (Fig. 1). The overall average of tuberculin-positive cattle was 3.55±0.75 years (Table 3). Frequency of pregnancy among tuberculin-positive cattle was 31.45% with the peak of 57.81% in 2018 (Table 4) with fluctuations and an overall increasing trend (Fig. 2). The average size of reaction to tuberculin inoculation was 7.21±2.70 mm. Map showcased that tuberculin-positive and suspected cases were present in all the cities.

Table 1: Coverage of tuberculin test screening program in Alborz Province, Iran between 2011 and 2021.

Year	City (N)							Total
	Eshtehard	Chahar Bagh	Savojbolagh	Taleqan	Fardis	Karaj	Nazarabad	
2011	7	9	52	7	13	24	90	202
2012	7	9	52	7	13	24	90	202
2013	7	9	52	7	13	24	90	202
2014	5	27	96	9	27	55	160	379
2015	6	18	101	7	23	61	160	376
2016	6	14	78	4	16	53	143	314
2017	7	17	75	2	21	47	158	327
2018	3	25	94	0	22	54	138	336
2019	6	28	99	5	26	46	118	328
2020	9	23	83	5	24	48	124	316
2021	9	18	70	1	24	52	108	281
Total	**72**	**196**	**852**	**54**	**222**	**488**	**1379**	**3263**

Table 1*:* Individual prevalence of tuberculin-positive cattle in Alborz Province, Iran between 2011 and 2021.

Year	Number of tested cattle	Number of positive cattle (%)
2011	81831	83 (0.101)
2012	95512	75 (0.078)
2013	83037	96 (0.116)
2014	168792	67 (0.040)
2015	179764	82 (0.046)
2016	162009	80 (0.049)
2017	180103	71 (0.039)
2018	212467	64 (0.030)
2019	188982	82 (0.043)
2020	176309	68 (0.039)
2021	169405	81 (0.048)
Total	**1698211**	**849 (0.050)**

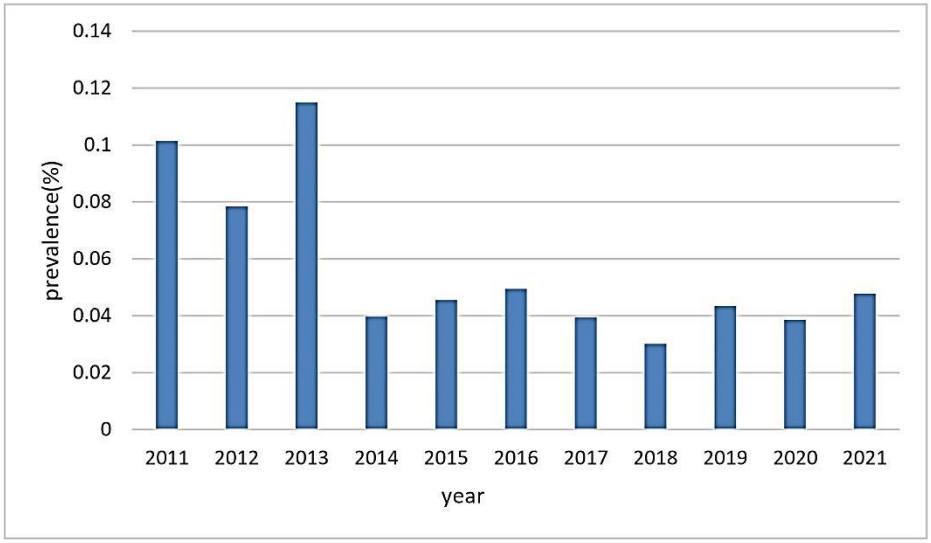

Figure 1: Trend of individual prevalence of tuberculin-positive cattle in Alborz Province, Iran between 2011 and 2021.

Table 2: Frequency of tuberculin-positive cattle based on age in Alborz Province, Iran between 2011 and 2021.

Year	Age (year)				
	2>	2-3	3-4	4-5	5<
2011	0	5	56	22	0
2012	1	18	25	27	5
2013	1	19	53	20	3
2014	0	11	45	9	1
2015	0	22	25	33	2
2016	0	20	33	26	1
2017	1	7	36	22	6
2018	0	11	39	12	1
2019	0	23	33	24	2
2020	0	19	27	19	3
2021	0	16	39	24	2
Total	**3**	**171**	**411**	**235**	**26**

Table 3: Frequency of pregnancy among tuberculin-positive cattle in Alborz Province, Iran between 2011 and 2021.

Year	Number of positive cattle	Number of positive pregnant cattle (%)
2011	83	24 (28.92)
2012	75	14 (18.67)
2013	96	32 (33.33)
2014	67	13 (19.40)
2015	82	20 (24.39)
2016	80	27 (33.75)
2017	71	18 (25.35)
2018	64	37 (57.81)
2019	82	27(32.93)
2020	68	21 (30.88)
2021	81	34 (41.98)
Total	**849**	**67 (31.45)**

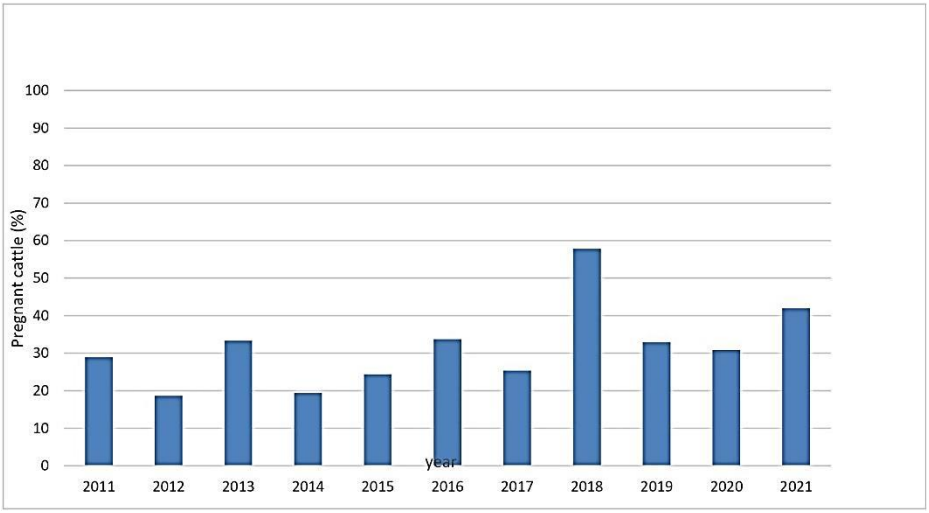

Figure 1: Trend of the relative frequency of pregnancy among tuberculin-positive cattle in Alborz Province, Iran between 2011 and 2021.

The highest overall individual prevalence of tuberculin positive and suspected cases was in Taleqan (0.1243%) and Chahar Bagh (0.0751%), respectively. Whilst Fardis (0.0676%), Savojbolagh (0.0491%), Nazarabad (0.0326%), Eshtehard (0.0283%) had lower values (Fig. 3).

Slaughterhouse condemnations

Among slaughterhouse condemnations, whole carcass condemnations, totalling 50 between 2011 and 2021, depicted an increasing trend from zero in 2011 to seven in 2021. Moreover, Mesenteric lymph node lesions (n=206) and pulmonary and laryngeal lesions (n=92) had the highest frequency of partial condemnation whilst septicaemia (n=1) and cervical and prescapular lymph node lesions (n=5) had the lowest frequency, respectively (Table 5). This study aimed to determine the screening coverage, individual prevalence, and the number of slaughterhouse condemnations in the cattle in Alborz province, Iran between 2011 and 2021.

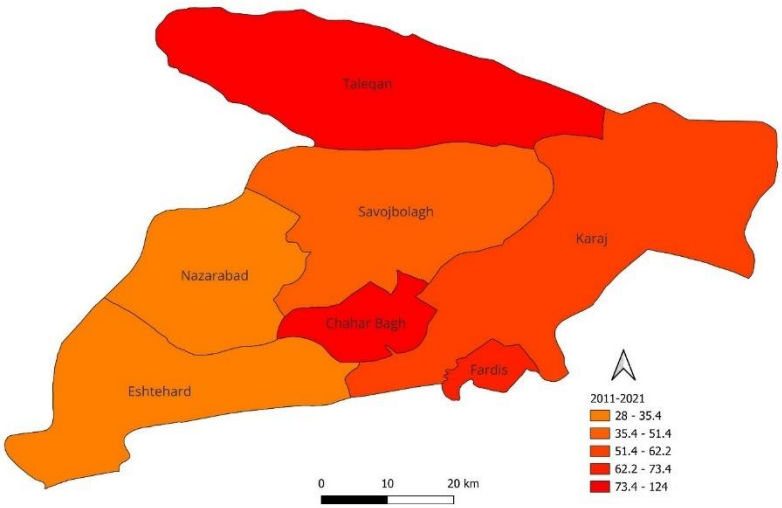

Figure 2: Frequency of tuberculin-positive and suspected cattle per 100000 cattle in Alborz Province, Iran between 2011 and 2021.

Table 5*:* Number of slaughterhouse condemnations in slaughterhouses in Alborz Province, Iran between 2011 and 2021.

Year	Type of lesion									
	Pulmonary and laryngeal lesions	Mesenteric lymph node lesions	Mediastinal lymph node lesions	Uterine lesions	Hepatic lesions	Diaphragm lesions	Cervical and prescapular lymph node lesions	Disseminated tuberculosis	Septicaemia	Kidney tuberculosis
2011	8	18	1	0	8	0	0	16	0	0
2012	10	11	2	4	0	4	0	3	0	0
2013	15	21	1	1	10	0	0	20	0	0
2014	12	26	2	0	15	0	0	5	0	1
2015	9	14	0	4	4	6	0	4	0	3
2016	5	21	0	3	5	10	0	2	0	5
2017	6	25	5	1	3	8	1	8	0	0
2018	10	15	2	0	8	2	1	10	0	0
2019	4	15	0	11	0	3	2	4	1	0
2020	7	20	0	0	2	13	1	1	0	2
2021	6	20	0	5	1	2	0	11	0	2
Total	**92**	**206**	**13**	**29**	**56**	**48**	**5**	**74**	**1**	**13**

Based on our findings, the coverage of tuberculin screening was not similar among the cities of Alborz province with the highest and lowest number of tested farms belonging to Nazarabad (n=1379) and Savojbolagh (n=852) and Eshtehard (n=72) Taleqan (n=54), respectively. The overall individual prevalence of bTB in cattle was 0.05% with an increasing trend between 2018 and 2021, which is inconsistent and lower compared with the study by Rabiee *et al*. (2016) where the average individual prevalence of bTB by tuberculin test in cattle was 15.7% (Rabiee *et al*., 2016). This could be due to the prevalence of

bTB being different in the provinces of Iran, which has been previously observed in Egypt, where the highest positive results for tuberculin skin test reactors was 22 out of 512 tested cattle (4.30%) in mid-delta, while the lowest value was 51out of 4170 tested cattle (1.22%) in Alexandria Road district (Hamed *et al*., 2021; Rabiee *et al*., 2016). This could be due to the role of wildlife reservoirs of bTB such as deer and badgers in the infection of cattle and lack of resources to compensate the farmer and as a result, inability in completely implementing the test-and-slaughter strategy to reduce the prevalence of bTB in cattle (Swift *et al*., 2021; Milián-Suazo *et al*., 2022).

However, Esmaeilzadeh *et al*. did not observe any clear and statistically-significant pattern of change of bTB in cattle in Khorasan Razavi province between 2007 and 2019 (Esmaeilzadeh *et al*., 2022). Zahrakar *et al*. conducted a cross-sectional study on 100 samples of raw milk collected randomly from dairy industrial and traditional cattle farms in 2020 in eight cities of Lorestan province, among which 26 samples (26%) were contaminated with Mycobacterium spp. and seven (7%) were contaminated with M. bovis (Zahrakar *et al*., 2023). In our study, the highest prevalence of tuberculin-positive and suspected cattle for 2011-2021 was observed in Taleqan (0.1243%), a city that has the lowest coverage compared to the other districts with only 54 cattle farms tested between 2011 and 2021. This inconsistency may warrant further investigations in Taleqan city.

The prevalence of bTB depicts a socioeconomic gradient by being focused in low- and middle-income countries, mainly affecting impoverished, marginalized, and rural section communities where people reside in close proximity with animals, with restricted access to sanitation, secure food products, and health care services (Kasir *et al*., 2023). Moreover, bTB is vastly unreported in these settings because of the inadequate public health surveillance (Toribio *et al*., 2023).

Along with M. bovis, other Mycobacterium tuberculosis complex members such as M. tuberculosis, M. africanum, M. caprae, M. orygis, and M. microti are recognized to be able to cause bTB in cattle (Ramanujam *et al*., 2023). This is one of the limitations of our study because although tuberculin skin test is approved by World Health Organization and World Organisation for Animal Health (O'Brien *et al*., 2023), its sensitivity and specificity is able to be altered by the strain of the Mycobacterium infecting the cattle, purified protein derivative products, the ability of the test performer in the injection of tuberculin and the measurement of the skin reaction, the age, and immune status of the screened animal (Sarkar *et al*., 2023).

In our study, the relative frequency of pregnancy among tuberculin-positive cattle was 31.45% with an overall increasing trend. This is consistent with the findings in Bangladesh where it was observed that the pregnancy of cattle increased the odds of bTB by 1.7 times

(95% CI: 1.2–2.4) compared to non-pregnant cattle (Islam *et al*., 2023).

In our study, whole carcass condemnation showed an increasing trend from zero in 2011 to seven in 2021 and among the partial slaughterhouse condemnations of the carcasses of the cattle, Mesenteric lymph node lesions (n=206) and pulmonary and laryngeal lesions (n=92) possessed the highest frequency. This is consistent with the findings of the slaughterhouse inspection of bTB in Portugal, where the most reported bTB legions were identified to be in the mediastinal and bronchial lymph nodes (Gonçalves *et al*., 2022). Moreover, in a study conducted in Italy, the detection of bTB in post-mortem inspection depicted an overall decreasing trend from 6.74% between 2010 and 2012 to 3.36% between 2017 and 2019 and a higher localization of lesions in the thoracic cavity was observed (Abbate *et al*., 2020).

The slaughterhouse inspection plays a pivotal role in the surveillance of bTB particularly in endemic settings by detecting the infected herds that are not subjected to tuberculin screening (Ramanujam *et al*., 2022). Also, due to concurrent infections in the herds such as Johne's Disease and Bovine Viral Diarrhea, exposure of the cattle to other non-pathogenic environmental mycobacteria, co-infection with Fasciola hepatica, and depressed cell-mediated immune response of the cattle, the sensitivity and specificity of diagnostic techniques in live cattle could be altered and slaughterhouse inspection is able to provide additional data and identify non-reactive cattle in these cases (Abbate *et al*., 2020).

Approximately, 10% of countries with the circulation of M. bovis in their cattle herds have implemented surveillance measures. However, most surveillance systems are passive surveillance and have not integrated into the One Health approach (de Macedo Couto *et al*., 2022). But, due to the bTB being a major public health concern throughout the world, particularly in Africa, Asia, and the Americas (Lema *et al*., 2022), the utilization of the One health approach to avert the outbreaks of bTB among animal and human populations whilst enhancing the food safety and security is recommended (Odetokun *et al*., 2022).

In conclusion, based on our findings, the total coverage of bovine tuberculosis screening coverage in the cattle in Alborz province, Iran between 2011 and 2021was the highest in Nazarabad (n=1379) and Savojbolagh (n=852) and the lowest in Eshtehard (n=72) Taleqan (n=54). And, the overall prevalence of bovine tuberculosis was 0.05% peaking at 0.11% in 2013 with an increasing trend between 2018 and 2021. The average age of tuberculin-positive cattle was 3.55±0.75 years. And, the average size of reaction to tuberculin inoculation was 7.21±2.70 mm. Moreover, no linear relation was observed between the positive number of cases and year and age categories of the infected cattle. The individual prevalence of tuberculin positive and suspected cases based on city was higher in Taleqan (0.1243%)

and Chahar Bagh (0.0751%), respectively. While Fardis (0.0676%), Savojbolagh (0.0491%), Nazarabad (0.0326%), Eshtehard (0.0283%) had lower values. Moreover, the relative frequency of pregnancy among tuberculin-positive cattle was 31.45% and depicted an overall increasing trend. Among, the abattoir condemnations due to bovine tuberculosis, 50 whole carcass condemnations were recorded with an increasing trend while among partial condemnations, mesenteric lymph node lesions (n=206) and pulmonary and laryngeal lesions (n=92) were found to have the highest frequency whilst septicaemia (n=1) and cervical and prescapular lymph node lesions (n=5) possessed the lowest frequency, respectively. Based on our findings, bovine tuberculin is present in all cities of Alborz province and more detailed investigations are recommended.

Conflict of interest

The authors declare that they have no conflict of interest.

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