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Epidemiological investigation of bovine tuberculosis infection dynamics in Turkey

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Abstract: A national epidemiological research project was carried out to define the dynamics affecting the epidemiology of bovine tuberculosis (bTB) infection in Turkey and to identify the risk factors. Official veterinarian (OV) and breeder original questionnaires were produced separately as part of this study to collect thorough data regarding the disease from the field. The number of questionnaires that needed to be filled out was decided by 95% confidence interval (CI) and 5% margin of error. The findings of 371 OV and 317 breeder questionnaires completed online across the country were analyzed. In addition, 28 outbreaks determined by random method were visited. To observe regional differences and field conditions, the opinions of OVs who monitored the disease and breeders were compiled. It was observed that the data obtained from both questionnaires was largely compatible. The main factors in the epidemiology of bTB infection were found to be effective fight, development of state policy, providing adequate financing, animal purchase, ear tagging and records, animal traders, animal markets, animal movements, conditional slaughtering, slaughterhouses, postmortem inspection, premise conditions, socio-economic impact, evaluation of raw milk, disease-free premises, compensation payments, and quarantine processes.

Key words: Questionnaire, *Mycobacterium bovis*, bovine tuberculosis, epidemiology, field survey

1. Introduction

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* (*M. bovis*) is an infectious chronic disease that affects a wide range of hosts, including cattle and humans [1, 2]. This highly adaptive and successful pathogen spreads worldwide. bTB remains a significant zoonotic infectious disease in cattle and other domestic and wild animals in many countries [3]. Despite all the efforts and measures taken for eradication over the years, the disease continues to pose a significant challenge in the global perspective. This zoonotic disease continues to exist and poses a threat to both animals' and public health [4].

The concept of "one health", which obscures the line between human and animal medicine, has long been recognized as a useful strategy for the control of zoonotic diseases [5]. bTB is a zoonotic disease of great concern and directly affects the cattle industry [2]. The prevalence of bTB has a negative socio-economic impact on public health, international trade, tourism, livestock, meat, and milk production [6]. bTB is a major cause of the economic loss of livestock owners worldwide, with an estimated >50 million infected cattle and an annual cost of $3 billion [7]. The socio-economic impact of bTB on public health and agriculture in Turkey is estimated to be between $15 and $59 million annually [8]. According to the data of the Ministry of Agriculture and Forestry (MoAF) in 2001, when this number was given, while 59 bTB outbreaks were reported in Turkey, this number increased to 2248[1] in 2019. As a result of tuberculin skin test (TST) positivity, 23,567 cattle were subjected to conditional slaughter.

[1] World Organisation for Animal Health (2019). World Animal Health Information System, Annual Animal Health Report in 2019 [online]. Website http://www.oie.int/wahis_2/public/wahid.php/ Countryinformation/reporting/reporthistory [accessed 29 August 2020].

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Approximately $15 million in compensation was paid to the breeders in return. The public health risk and socio-economic impact are thought to have increased a result of the rise in bTB disease prevalence accordingly [9].

Separate questionnaires were prepared for the official veterinarians (OVs) who monitor the disease in the field and the breeders who have bTB in their premises, and their responses were gathered in this study to determine the epidemiological reasons for Turkey's alarming increase in the number of bTB outbreaks in recent years. In addition, to observe the regional differences and conditions in the field, premises with outbreaks determined by random method were visited on-site. The reasons for the increase in epidemiological terms were revealed with two-way confirmation by carrying out interviews with the OVs who monitored the disease and breeders. The goal of this study was to determine national field facts about the epidemiology of bTB disease in order to develop disease control strategies.

2. Materials and methods

2.1. Preparation of questionnaires

The questionnaires were prepared based on the literature on the disease and experience in the field. The approach developed by Ciaravino et al. (2017) was used to investigate the perceptions, opinions, attitudes, and beliefs of veterinarians and farmers who determined the effectiveness of the Spanish bTB eradication program [10]. Two separate questionnaires were prepared, one for OVs who monitor bTB disease in the field and the other for the breeders who have bTB disease in their premises. It was made clear that the responses of the participants would only be used for scientific purposes, and that confidentiality would be maintained. The participants were not required to fill out the form using their first and last names. Participation in the research was completely voluntary. They might choose not to participate in the study, or withdraw if they did not wish to answer questions. The real reasons for the prevalence of bTB disease in the field were first-hand anonymously questioned through questionnaires. For this purpose, the participants were asked fill in the blanks, multiple choice, sequential, closed, open-ended, guided by the answer they gave to the previous question, and cross questions. The validation of the questionnaires was provided by a preliminary field trial. The answers given to the cross questions in both questionnaires were found to be significantly correlated with the chi-square test statistic at the 0.05 significance level.

2.2. Pretesting the questionnaires

After the questionnaires were prepared, they were shared with the researchers involved in the national project we conducted, and required modifications were made based on their feedback. The questionnaires were pretested by interviewing the breeder in a premises with a bTB outbreak in the Kahramankazan district of Ankara and the OV that monitored the disease in this premises. It was concluded that the questionnaires were applicable in the field. They were then transferred to the electronic environment to reach a wider target audience and to obtain data more easily. The link 'https://docs.google.com/forms' was shared and opened for online data entry by Provincial/District Agriculture and Forestry Directorates (PDAFD).

2.3. Selection of participants

The OVs in Turkey had a target population of 6543 people registered in the Veterinary Information System (VETBIS) of the MoAF, working in the fight against animal diseases in the 81 PDAFD. Based on this number, the number of OVs to be included in the research was determined by a two-stage random sampling method. The number of OVs working in each province was examined in the first stage, and the number of active bTB outbreaks in these provinces between January 1, 2017 and March 6, 2020 was considered in the second stage. The minimum total number of included OVs in Turkey was calculated as 363 using a 95% confidence interval (CI) and a 5% margin of error (http://www.winepi.net).

The target population of the breeders was the cattle breeders in Turkey. The source of the target population was VETBIS. The number of included breeders was determined by a two-stage random sampling method in 66 provinces with 752 active bTB outbreaks that occurred between January 1, 2017 and March 6, 2020. The selection criteria of the breeders to be included were the districts where the bTB outbreaks were reported in the first stage and the villages where the outbreaks occurred in these districts in the second stage (https://stattrek.com). The minimum total number of breeders in Turkey was calculated as 255, considering 95% CI and a 5% margin of error out of 752 outbreaks determined between the mentioned dates (http://www.winepi.net).

2.4. Selection of sites for epidemiological field survey

A total of 28 settlements were determined for the epidemiological field survey with fourteen provinces from seven regions of Turkey and two settlements (back-up) from each province. The numbers and settlements were chosen at random by considering the criteria like geographical region, bTB outbreak density in the province, accessibility, and the project budget. VETBIS and Animal Registration System (TURKVET) of MoAF were used to determine the premises with an outbreak to be visited in this settlement. In the field research, opinions of OVs and breeders on the epidemiology of bTB disease were taken and on-site observation was made.

3. Results

3.1. The number of participants

371 OVs from 72 provinces across Turkey and 317 breeders from a total of 61 provinces, 59 from designated provinces and 2 from other provinces answered the questionnaires. In this context, the numbers included both in the OV and breeder questionnaire exceeded the numbers calculated using 95% CI and 5% margin of error. The reason for this was that the questionnaire access link remained available between the dates specified in the official letter, and other premises with a bTB outbreak participated in the breeder questionnaire in order to determine the epidemiological situation in the premises in the same settlement. All of the questionnaire responses were taken into account.

3.1.1. Findings of official veterinarian questionnaire

As shown in Figure 1, 84.4% of the OVs working in the field were young staff with less than 15 years of experience.

As can be seen in Figure 2, on a provincial basis, there was at least one bTB outbreak at a rate of 76.3% in the last 6 months. The presence of 72% of active bTB outbreaks the field indicates that the spread of the disease continues.

The long and costly quarantine period, trade restrictions of livestock and milk, and restriction of pasture usage of positive premises were reported as the first three reasons for breeders to avoid reporting disease.

OV pointed out the importance of slaughterhouses as a milestone in public health and disease control, since postmortem inspection of cattle in slaughterhouses revealed 64.7% of bTB.

The purchased infected animal was the main transmission route of the disease on premises. Undocumented animal movements, animal markets, and animal traders' movements were reported by OVs as the leading mediating factors in spreading the disease. It was understood that 66% of road controls made by law enforcement officers to prevent undocumented animal movements were not properly carried out.

In premises with a bTB outbreak, the median value of the number of animals conditionally slaughtered from the onset of the disease until its extinction was determined as 35%. OVs declared that the time for animals to be sent to conditional slaughter was approximately 16 days. It has been stated that the period of animals sent to be slaughtered was extended due to the problems in price research and finding suitable slaughter places. The compensation of the animals conditionally slaughtered due to bTB positivity was paid to the breeders in 3 months at a rate of 64.4% according to questionnaire results.

OVs reported that bTB disease lesions were detected at a rate of 1%–2% in slaughterhouses and sacrificial slaughters. According to OVs, ear tagging and recording of animals that have already been tagged with permanent and noninterventable tools to trace the origin premises (up to 6 months) is important in terms of combating the disease. It was indicated by OV that the change in the regulation called "ear tagging amnesty", the deterioration of traceability for needs such as bank credit and income statement was enough to nullify the fight against all epidemic diseases.

OVs stated that classifying livestock premises was required to improve the conditions in terms of animal health and welfare. The increasing number of disease-free zones, premises, and animals was reported to be important in terms of both healthy breeder supply and healthy meat and milk production. OVs responded positively to a vaccine that provides immunity around 70% at a rate of 89.2% (Figure 3).

The vast majority of OVs stated that delivering blood serum to the laboratory for the Interferon-gamma (IFN-γ) test within 8 h would be challenging. However, this test could be used in some special cases. A rate of 74.7% of OVs in Turkey thought that wildlife had no effect on the transmission of bTB.

74.1% of OVs stated that they did not find the program implemented by MoAF in the fight against bTB disease

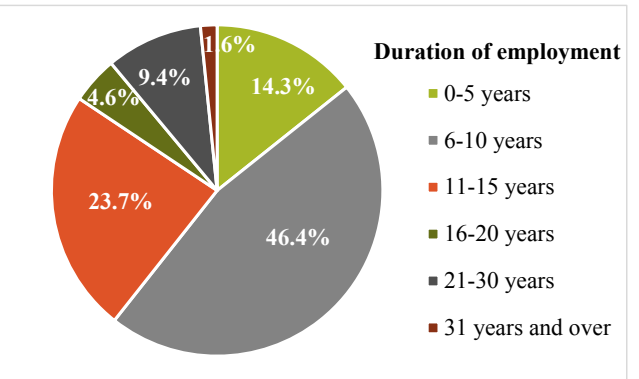

Figure 1. Total employment duration of official veterinarians.

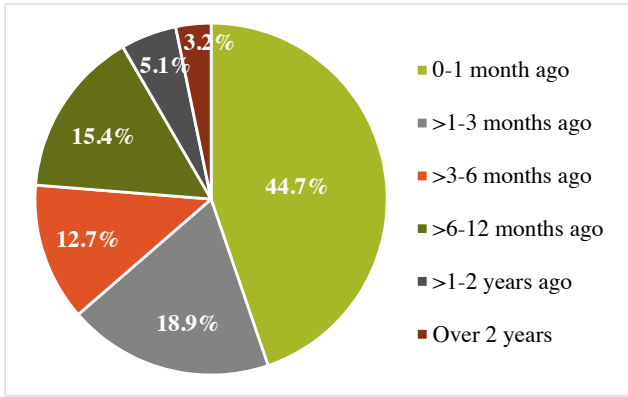

Figure 2. The last time the bTB outbreak was reported.

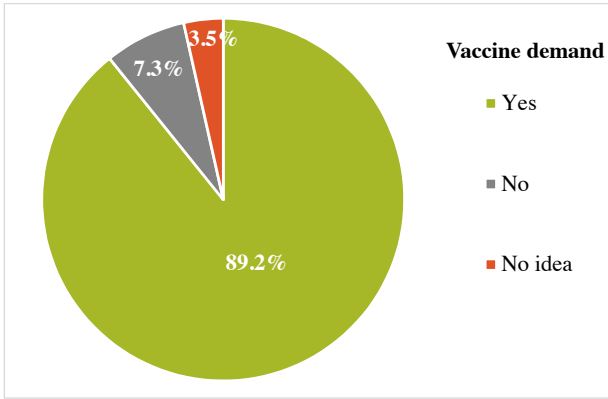

Figure 3. The percentage of vaccine demand by official veterinarians.

sufficient or partially sufficient. OVs emphasized the importance of developing a national policy to combat the disease and of controlling animal movements with the participation of all stakeholders, as shown in Figure 4, which includes recommendations for control and eradication in the fight against bTB disease.

3.1.2. Findings of breeder questionnaire

As shown in Figure 5, 51.7% of the breeders in Turkey were primary school graduates, followed by high school and secondary school graduates.

77% of the premises made mixed production, followed by milk, fattening, and breeder type production, respectively. It was determined that these premises were generally close to each other, 51.7% of them were semiopen, 42.9% of them were closed, and the median value of the number of animals per premise was 22 heads.

It was reported by the breeders that bTB disease was detected at a rate of 90.5% in the slaughterhouse or after sacrificial slaughter. 85.5% of the premises did not have bTB disease before. In premises with bTB outbreaks, the median percent value of the number of animals sent to conditional slaughter and died in the period from the onset of the disease to its extinction was calculated as 39%. The price range and proportional distribution of the economic loss of the premises where the bTB disease originated is shown in Figure 6.

Raw milk collected from bTB negative animals in quarantine was mostly heat-treated and used.

According to breeders, 94.6% of the bTB-positive animals were conditionally slaughtered within 1 month (approximately 17 days on average) and 68.1% received compensation within 3 months. While there was no change in the decision of 76% of the breeders if the compensations were not provided on time, it was discovered that the decision of 24% of the breeders was affected by this situation.

The breeders stated that 38.2% of the neighboring premises did not report any suspected cases of bTB. The first three reasons for this situation are the long time taken for quarantine and bureaucratic procedures in the fight against the disease, not selling animals and milk, and not receiving compensation on time. As shown in Figure 7, at least 45.1% of the breeders reported bTB disease in other premises in the same location.

As depicted in Figure 8, there was a 64.7% rate of animal entry to the premises in a calendar year. The rates

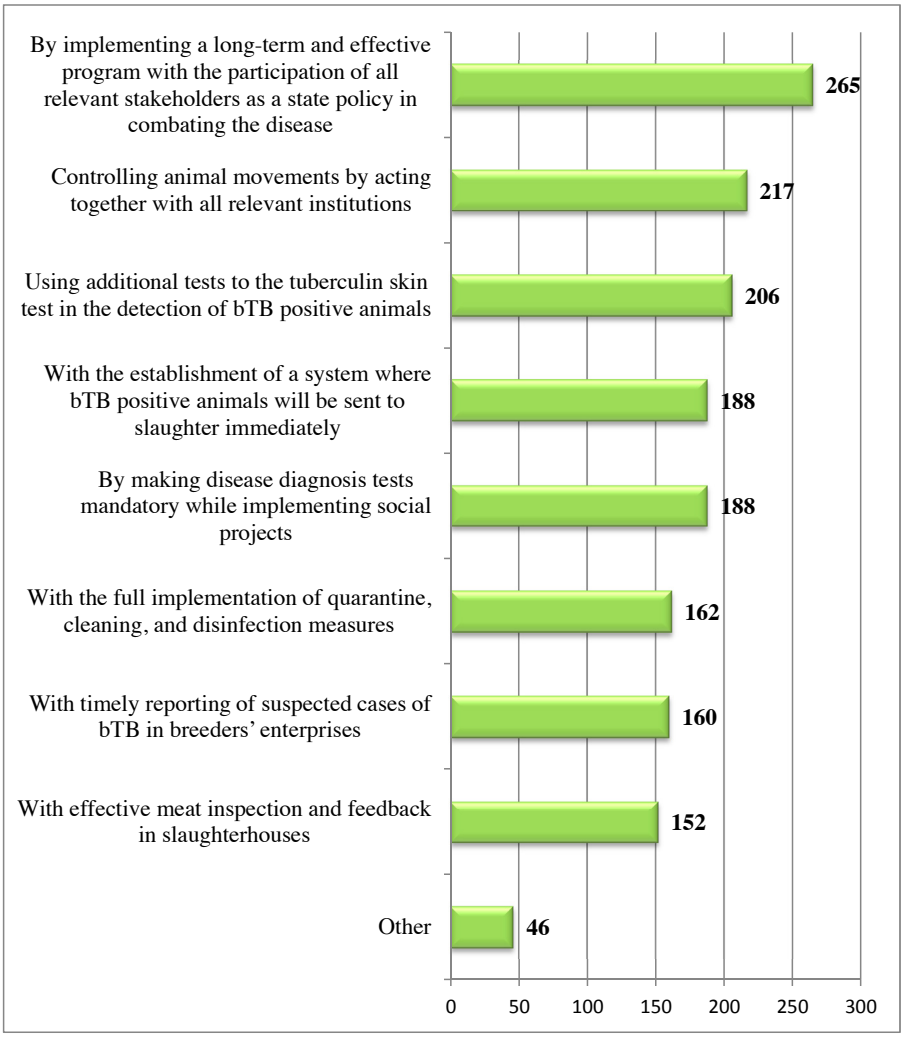

Figure 4. The recommendations of official veterinarians for the control and eradication of bTB disease.

of animal introduction to the premises before the disease outbreak were also found to be similar.

It was understood that the detection of bTB disease lesions in sacrificial slaughters was between 1% and 2%. Of the breeders, 97.2% stated that they would like to prefer to use the bTB vaccine that could be developed. According to the breeders, the wild animals on the pasture did not have a major effect on the transmission of the bTB disease.

According to the breeders, the fight of MoAF against bTB disease was sufficient and partially sufficient at a rate of 86.7%. The suspected cases not reported on time, the ineffectiveness of the implemented control program, and the inability of the tests to fully detect sick animals are among the first three reasons for the inadequacy of MoAF in the fight against bTB disease. As shown in Figure 9, the first three measurements to be taken in the fight against bTB disease were determined as the implementation of a long-term and effective program as a state policy with the participation of all stakeholders, the development of tests, and the prevention of undocumented animal trading.

3.2. Findings of the field survey

Despite the risk of COVID-19 disease, premises owners with outbreaks and OVs that monitor the disease in these premises were visited for the epidemiological field survey in fourteen provinces across seven regions of Turkey, in a total of 28 settlements, 26 of which were randomly determined and two substitutes. Information on the epidemiology of bTB infection was compiled during these interviews and on-site observation.

3.2.1. Findings of official veterinarian field survey

According to OVs, postmortem inspection of cattle carcasses in the slaughterhouse resulted in the detection of bTB disease in more than 90% of cases. It was stated that infected animal purchases and the movements of the

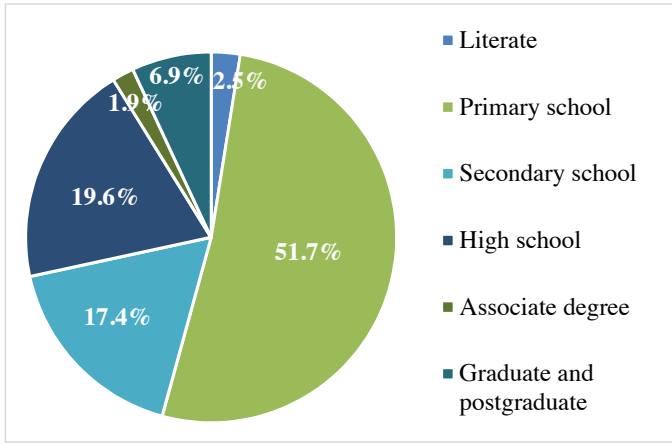

Figure 5. Education status of the breeders

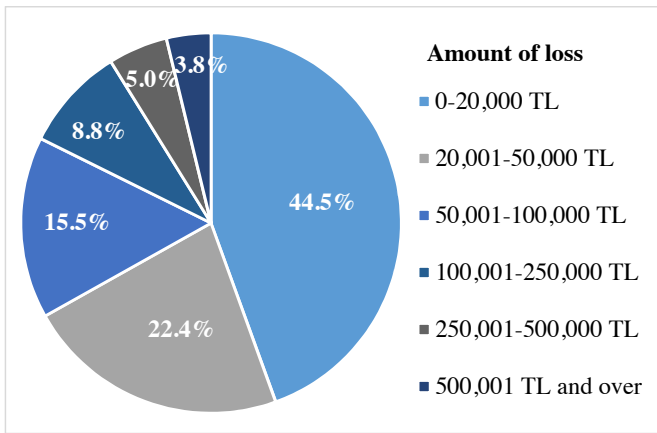

Figure 6. Proportional distribution of econonic loss of the premise caused by the bTB disease (1$ is approximately 8.5 Turkish Lira)

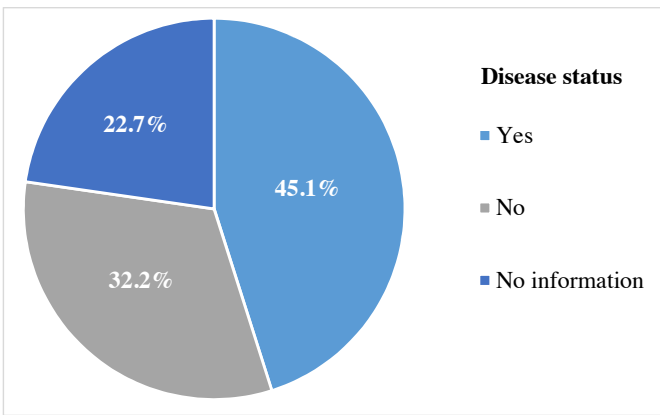

Figure 7. bTB disease in other premises in the same location.

traders were the first two places where the disease was introduced, and animal movements and animal markets were the main factors in the spread of the disease.

It was reported that the animal stock in premises with bTB disease was generally out of date. Furthermore, the changes in the regulation known as "ear tagging amnesty"

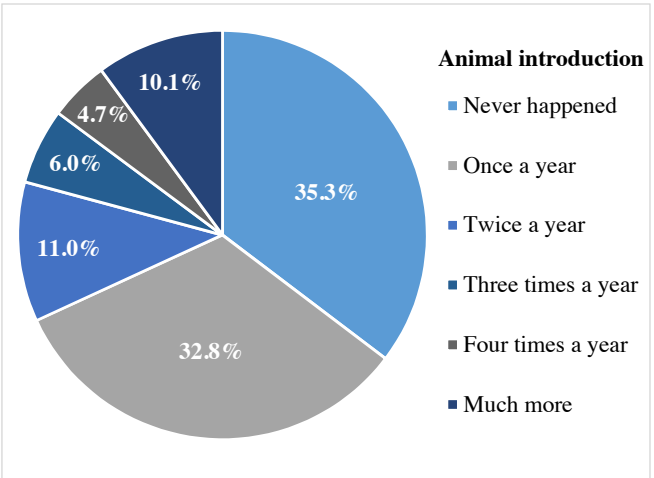

Figure 8. The frequency of animal introduction to the premise.

pose a problem. The ear tags of the animals should be permanent and their records should be traceable.

It was stated that the conditional slaughter should be done by the Meat and Milk Institution (MMI) instead of private slaughterhouses. It was reported that bTB-positive animals were slaughtered in about 16 days. Furthermore, it was stated that an average of 45% of the animals in the premises were slaughtered between the emergence of the disease and its extinction. It was also reported that compensation was paid to the breeders in an average of 67 days. The critical role of paying compensation on time and regularly was also reported.

According to the report, bTB disease lesions were detected in approximately 1% of animals slaughtered in slaughterhouses after postmortem inspection and 0.33% of sacrificial slaughters. However, bTB positivity rate was estimated to be higher in cases where general TST screening was performed.

While bTB disease is generally more common in dairy farms and in elderly cows, it has been declared that it is less common in fattening farms and that the conditions of livestock premises should be improved by classifying them. Due to time constraints, the IFN-γ test was reported to be challenging to use in the field.

It was stated that studies on progressive disease-free areas can be implemented regionally, and that the premises had problems in finding disease-free breeding animals. Furthermore, it has been reported that tested or disease-free breeding animals should be distributed through social projects.

OV emphasized that the disease fight should be carried out as a single practice throughout the country, with the spirit of mobilization as a state policy and in coordination with all relevant institutions. Furthermore, OV stated that it is necessary to focus on creating teams that are fully equipped and free from unnecessary workload to intervene in diseases in the field. In addition, it was stated that there was an intense demand for the development of a vaccine against bTB.

3.2.2. Findings of breeder field survey

The breeders declared that the detection of bTB disease was determined by postmortem inspection in the slaughterhouse at a rate of 90%. It was reported that the possible transmission route of the disease to the premises was the newly purchased infected animals, followed by uncontrolled movement of traders.

It was stated that undocumented animal movements were made for various reasons. It was declared that some breeders avoid reporting for various reasons, such as cuts in payments, long quarantine periods, and unawareness. It was reported that the disease persists for a long time in the form of focci in some settlements. It was declared and observed on side that generally the conditions of livestock were not good in terms of physical, health, and welfare, and the premises were close to each other.

It was stated that there were problems with the slaughtering, transportation, and payments of the bTB positive animals in the MMI slaughterhouses. These animals were sent to slaughter in about 15 days, the compensation was paid in an average of 92 days, and an average of 55% of the animals on the premises were sent to slaughter from the onset of the disease until its extinction.

bTB disease lesions were found in approximately 2% of sacrificial slaughters. The loss due to bTB disease was estimated to be around 105,000 TL per premises, and it was reported that milk sales in dairy farms decreased by approximately 90%, and the loss in some premises affected the family's livelihood.

Breeders suggested that studies be conducted for progressive disease-free areas, that breeding animals be

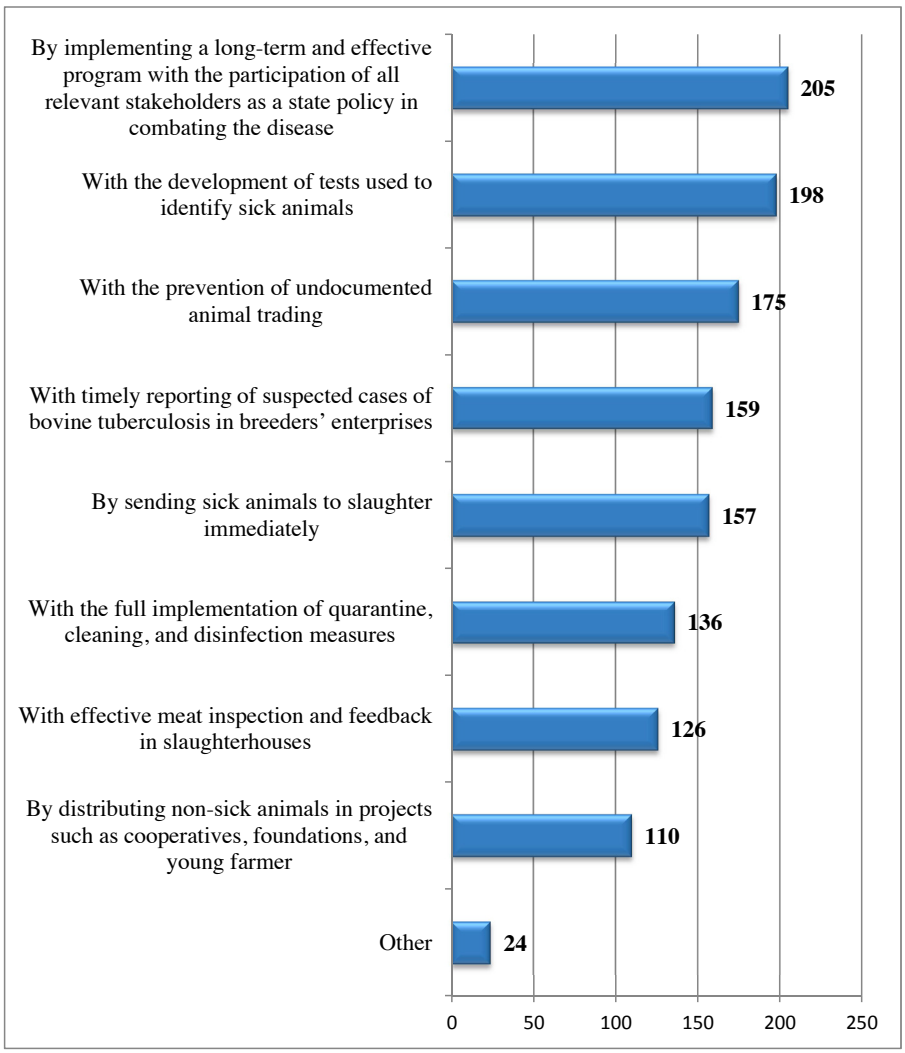

Figure 9. Necessary measures to be taken by breeders in the fight against bTB disease

Table. Comparison of questionnaires and epidemiological field survey values

Topics	Questionnaire		Field survey	
	OV	Breeder	OV	Breeder
Detection of bTB disease (Slaughterhouse-sacrificial slaughter)	64.7%	90.5%	> 90%	90%
Time to send to the conditional slaughter (approximately days)	16	17	16	15
Time to pay compensation to breeder (days)	90	90	67	92
Median percentage of animals sent to slaughter	35	39	45	55
The incidence of bTB lesions in slaughterhouses	1%–2%	-	Approx. 1%	-
The incidence of bTB lesions in sacrificial slaughter	1%–2%	1%–2%	Approx. 0.33%	Approx. 2%

purchased from disease-free premises, and that TST be implemented on dairy-breeding farms.

The breeders also stated that they were generally satisfied with MoAF's efforts to combat the disease. However, they also mentioned the need for training of breeders. They also stated that raw milk is generally given to companies that apply heat treatment. Furthermore, according to breeders, the younger generation does not

want to breed livestock. They also stated that there was a high demand for vaccine development, and there was no suspected case of bTB from wildlife.

Table shows the common values reported as a result of the questionnaires and field survey for comparison.

4. Discussion

Despite the fact that several studies on bTB disease have been carried out in Turkey to date, no research on the epidemiology of the disease at the national level has been found in the literature review. The last survey was conducted in 2857 herds in 2011 as part of the European Union project "Eradication of Brucellosis and Tuberculosis in Turkey," in which the Dutch government was a partner. The individual prevalence was 1.4% (95% CI) and the herd prevalence was approximately 2.5% (95% CI) with the comparative intradermal tuberculin skin test (CITT) [11]. According to the World Organization for Animal Health (OIE) Turkey reports, while the number of bTB outbreaks was 348[2] in 2011, this number was reported as 2248[3] in 2019.

In this context, this research was compiled for the first time in Turkey at the national level, by using the epidemiological data reports of the disease obtained from the systems, the prepared OV and breeder questionnaires, and the opinions of the OVs and breeders by visiting the premises with outbreaks. This research was planned with broad participation on a national scale. In this study, the opinions of the OVs and breeder questionnaires, and the findings of the field survey were compared, and the results were found to be largely compatible with each other.

When the obtained data was evaluated, it has been understood that the main factor in the introduction of bTB disease to the herd was the purchase of infected animals. The movements of traders and animal markets were shown to be important in the spread of the disease and outbreaks were active in the field.

Since approximately 90% of the disease was detected after postmortem inspection in the slaughterhouse, it has been understood that postmortem inspection surveillance of the slaughterhouse is the cornerstone of the fight against the disease. In premises with bTB disease, the time to be sent to conditional slaughter was approximately 2 weeks, and the time to pay compensation to the breeder was approximately 3 months. It was declared that the rate of being sent to the conditional slaughter from premises with outbreaks was in the range of 35%–55% from the onset of the disease to its extinction.

It was declared and observed that the loss caused by the bTB disease and the decrease in milk sales, especially on dairy farms, even affected the livelihood of the family. The rate of bTB positivity in general TST screening was expected to be greater than the rate of bTB lesions detected in slaughterhouses and sacrificial slaughters, which was estimated to be 1%–2%.

It was stated that by classifying the livestock premises, it was necessary to improve the ones whose conditions were not suitable in terms of animal health and welfare. The implementation of disease-free studies was important for the supply of healthy breeders. It was observed that there was intense demand for the development of vaccines to fight against the disease.

Controlling animal movements with the participation of all stakeholders, developing a state policy and effectively combating bTB disease by providing adequate and sustainable financial support were expressed as the common opinions by the participants.

The goal in animal production should not be intensive production, but healthy production in which the issue of animal health is focused.

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