Re-Emerging Bovine Tuberculosis in Northern Israel: Overview and Outbreak Description in a Family Dairy Farm

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ABSTRACT

Bovine tuberculosis (bTB), caused largely by *Mycobacterium bovis*, is a chronic infectious disease of cattle with significant animal health, economic, and public health implications. As a zoonotic pathogen, *M. bovis* can infect humans through direct contact with infected animals or consumption of unpasteurized milk and dairy products. Despite the implementation of national control and surveillance programs, bTB persists in many regions worldwide, often in association with wildlife reservoirs, posing ongoing challenges to eradication efforts. This article describes a bovine tuberculosis outbreak in a small family-operated dairy farm in Moshav Prazon, located in the Jezreel Valley in northern Israel. The event was first identified following the sudden death of a 22-month-old heifer with a history of recurrent pneumonia. Post-mortem examination revealed disseminated granulomatous lesions affecting the lungs, liver, spleen, peritoneum, and diaphragm. Diagnostic investigation at the Kimron Veterinary Institute confirmed the diagnosis of bovine tuberculosis by *M. bovis* infection using direct acid-fast staining, PCR, mycobacterial culture and histopathology. An epidemiological investigation was conducted alongside herd-level diagnostic testing using the Comparative Tuberculin Skin Test (CTST) and Interferon-Gamma Assay (IFNɣ). Among 81 cattle tested, 45 animals (56%) were positive in at least one diagnostic test, indicating a high within-herd prevalence. Based on these findings, and in accordance with national guidelines, the entire herd was depopulated. At slaughter, tuberculosis-suspect lesions were identified in 51% of animals (49 out of 96), and *M. bovis* was confirmed by PCR in a substantial proportion of sampled tissues. Agreement between diagnostic methods ranged from fair to substantial, as anticipated in the literature. All dairy herds within a 3-km radius were found negative for bTB by CTST. Four different former outbreaks in dairy farms in the same region and serological evidence of *M. tuberculosis*-complex exposure in wildlife in this region suggest a possible role of wildlife in the epidemiology of re-emerging bTB in Israel. This case, and the four previous outbreaks in Beit She'an valley area within six years, highlight the complexity of bTB control, the limitations of individual diagnostic tools, and the importance of integrated holistic surveillance of dairy farms and wildlife.

Key Words: Bovine Tuberculosis; Reactor Animal; Tuberculin; CTST; Gamma Interferon; Granuloma.

INTRODUCTION

Background

Tuberculosis is one of the leading bacterial causes of mortality worldwide. It is estimated that more than one-third of the global population is infected with *Mycobacterium tuberculosis*, and between 1.5 and 2 million people die from tuberculosis each year (1). Three main forms of tuberculosis affect different species: human, avian (poultry), and bovine tuberculosis (2).

While *Mycobacterium tuberculosis* remains the primary causative agent of human tuberculosis, the principal zoonotic form of the disease is caused by *Mycobacterium bovis*, which is capable of infecting both humans and animals (3). *M. bovis* is estimated to account for approximately between 3-15% of human tuberculosis cases worldwide (4, 5). Bovidae species and humans may become infected via aerosol transmission through droplets exhaled or coughed up by infected animals (6). Nevertheless, the main route of transmission from cattle to humans is the consumption of unpasteurized milk and dairy products (7). Livestock caretakers and veterinarians are particularly exposed to infectious droplets, especially in enclosed facilities (8). Transmission via contaminated pastures, water, or fomites is considered secondary to respiratory spread (9). Bacteria-containing saliva may further disseminate within the farm environment through shared drinking troughs (10).

Pathogenesis

Infection with *M. bovis* generally occurs when cattle inhale aerosolized tubercle bacilli, potentially even a single bacillus contained within a droplet nucleus (11). These droplets lodge in the respiratory tract, often reaching the alveolar surface of the lungs (12). The bacilli are phagocytosed by alveolar macrophages, which then interact with cells of the innate and adaptive immune systems within the affected tissue or draining lymph nodes (13). However, *M. bovis* resists intracellular destruction by inhibiting phagosome maturation and acidification, allowing the bacteria to survive and replicate within macrophages (14).

In response to persistent infection, immune cells, including activated macrophages, T lymphocytes, and later multinucleated giant cells, are recruited to the site of infection (14). The accumulation of these cells leads to the formation of a granuloma (tubercle), a highly organized structure aimed at containing and controlling the infection. Most bacilli remain localized in the upper respiratory tract after inhalation and contribute to the development of tubercles, which are characteristic lesions of bovine tuberculosis (14).

Although the granuloma's function is to contain the pathogen, they may also serve as a niche in which *M. bovis* persists and continues to replicate. In tuberculosis, a tubercle is a granulomatous lesion composed of epithelioid macrophages and Langhans giant cells surrounding central caseous necrosis, reflecting chronic cell-mediated immune containment, in contrast to abscesses, which are acute suppurative inflammations characterized by neutrophil accumulation and liquefactive necrosis (15, 16), and are less common in TB. Tuberculous lesions commonly detected at slaughter are firm, round, yellow-gray nodules measuring 2-20 mm or more in diameter, primarily in the lungs and lymph nodes (17), but may also be present at a later stage, where the nodule contents are liquefied and rich with mycobacteria, thus posing a significant occupational risk to abattoir workers.

Experimental infections in calves have shown that following a lag period after inoculation with *M. bovis*, bacteria can be consistently isolated from nasal mucus. Subsequently, bacterial shedding becomes intermittent, with the quantity and frequency of shedding appearing to be related to the infectious dose (11).

Transmission

Mycobacteria can be transmitted through infected secretions, including milk, vaginal discharge, urine, semen, feces, saliva and exudates from lesions. When the respiratory system becomes involved, particularly as the disease progresses to advanced stages with more extensive lesions, the risk of transmission increases (18). Both animals and humans may become infected through direct contact with contaminated skin wounds, ingestion of contaminated material, or inhalation of infectious aerosols (19). To establish infection via ingestion of contaminated material, a substantially higher bacterial load is required compared with inhalation (20). Adult cattle are most infected by Mycobacteria species via the aerosol route, whereas calves are frequently exposed through ingestion of contaminated milk (21). Sexual and congenital transmission are possible but occur less commonly (22). Human-to-human TB transmission primarily occurs via the respiratory route (23). However, transmission efficiency of bovine tuberculosis is considerably higher from cattle to cattle than from cattle to human (24).

Mycobacterium species are capable of surviving in the environment for several months, particularly under cold, humid, and dark conditions, which may facilitate indirect transmission (25).

Clinical Signs

Tuberculosis in cattle is generally a chronic, slowly progressive disease. In the early stages, infected animals are typically asymptomatic; however, as the disease advances, clinical signs may include weakness, low-grade fever, progressive emaciation, decreased appetite, and reduced milk production (26). Because lesions are most commonly located in the upper respiratory tract, lungs, and tonsils, respiratory signs such as dyspnea, moist cough, and tachypnea may be observed (27). The disease course may extend over months or even years before clinical manifestation, although in some cases it may present as an acute condition with rapid progression (28).

Human tuberculosis is likewise a chronic and debilitating disease. It often begins without apparent symptoms and gradually progresses to include decreased appetite, weight loss, and nocturnal sweating. When pulmonary involvement occurs, clinical signs such as dyspnea, chest pain and tachycardia may develop (29).

DIAGNOSIS AND METHODS

Clinical Diagnosis

Clinical indicators include weakness, low-grade fever, wet cough and swollen lymph nodes (30). Because its clinical signs are non-specific and can take months to years to become apparent, bovine tuberculosis is rarely diagnosed on clinical grounds.

Post mortem Diagnosis

Evidence of bovine tuberculosis in a herd is optimally gained through routine *post-mortem* investigation, especially if they present emaciation and history of cough, weakness and respiratory distress. The cornerstone for *post mortem* TB diagnosis is finding typical tubercles. Those pathological alterations can be detected in the lymph nodes, especially around the head, neck, chest and abdominal organs (26). These granulomas have usually a spherical yellowish caseous appearance, sometimes calcified, and are frequently encapsulated (31), but can also be presented in various maturation stages, and sometimes enlarged lymph nodes, easily overseen, are evidence of initial forms of TB.

Comparative Tuberculin Skin Test (CTST)

The CTST is an effective epidemiological and diagnostic tool for managing tuberculosis in cows (32). The TST involves injecting a dose of 0.1ml *M. bovis* Purified Protein Derivative (PPD) intradermally mostly in the neck area, followed 72 hours later looking for swelling (sign of delayed hypersensitivity) at the injection site (33). The procedure is demonstrated in Figure 1. For comparative TST (CTST) we use as control, a *M. avium* PPD, which is injected beside it at the same time. The protein solution found in the cell walls of *Mycobacterium* species is utilized to produce tuberculin (34). The injection of this protein solution into the skin of an infected animal triggers a local inflammatory reaction by the body's sensitized immune system, which results in a positive tuberculin test and the animal is then called a "reactor" (35). An animal who has not been exposed to bovine tuberculosis will not show an inflammatory response following the tuberculin injection.

Interferon gamma (IFNɣ) release assay (IGRA)

Blood samples from cattle infected with bovine tuberculosis can be used to measure the IFNɣ level released upon exposure of blood lymphocytes to PPD *in vitro*, and serves as an additional diagnostic tool. Previous exposure to *M. tuberculosis*-complex bacteria, such as in infected animals, leads to increased IFNɣ release following *in vitro* stimulation (36). This method was developed for TB diagnosis in the 1990's and accepted for bTB diagnosis by the World Organization for Animal Health (WOAH) in 2015. It has been integrated into the bTB diagnosis setup of the Veterinary Services and Animal Health (IVSAH) since 2019.

Bacterial identification

The "gold standard" for confirming infection remains mycobacterial isolation via culture, which is essential for downstream molecular typing and epidemiology. However, identifying *M. bovis* through culture and biochemical methods is challenging due to its fastidious nature and its exceptionally slow growth (37), necessitating high-level biosafety laboratories and trained personnel. Cultures are

Figure 1. Comparative Tuberculin Skin Test (CTST) procedure.

often heavily contaminated despite available decontamination procedures, which rely on the acid-fast resistance inherent to mycobacteria. Nucleic Acid Amplification Tests (NAATs), such as PCR, serve as a rapid alternative to bacterial isolation. The advantages of NAATs over culture include faster turnaround times, higher throughput, and increased safety. Yet, first-line NAATs often struggle to differentiate between *M. tuberculosis*-complex (MTBC) species and are prone to reduced sensitivity due to PCR inhibitors. Ultimately, the sensitivity of both methods is highly impaired by the paucibacillary nature of TB lesions throughout most stages of the disease.

EPIDEMIOLOGY

Global Distribution of Bovine Tuberculosis

Bovine tuberculosis is present worldwide, with the exception of Antarctica (38). However, due to the implementation of effective eradication programs, several countries are currently classified as free of bovine tuberculosis. These include Australia, Sweden, the Czech Republic, Norway, Austria, Switzerland, Luxembourg, Jamaica, Latvia, Slovakia, Iceland, Estonia, Canada, Lithuania, Finland, Barbados, Denmark, and Singapore (39, 40). In addition, ongoing initiatives aimed at eradicating bovine tuberculosis are being implemented in the United States, New Zealand, Japan, and several European countries (40, 41). In the last 10 years, only the United Kingdom, Ireland and Spain reported a prevalence higher than 1% in EU members and UK (42). It is important to note that formal "freedom" of disease does not necessarily imply that the disease, or sub-clinical infections, are completely absent in a country. In fact, for purposes of formal bTB "freedom" status by the WOAH, a non-zero prevalence in non-imported livestock below 0.2% is acceptable (43). This is mainly due to the highly imperfect diagnostic tests available, the usual lack of clinical and pathological manifestation of infection, and the possibility of wild-life reservoirs.

Incidence in Israel

The incidence of human tuberculosis in Israel has declined over recent years. In 2024, the reported infection rate was approximately 3 cases per 100,000 population (44). There are no reports of bovine tuberculosis zoonotic transmission in Israel. Between 2018 and 2023, thirty-eight *Mycobacterium bovis* isolations from humans were reported; all associated with prior BCG vaccination rather than transmission from animals (Dr. Perl, personal communication, Ministry of Health).

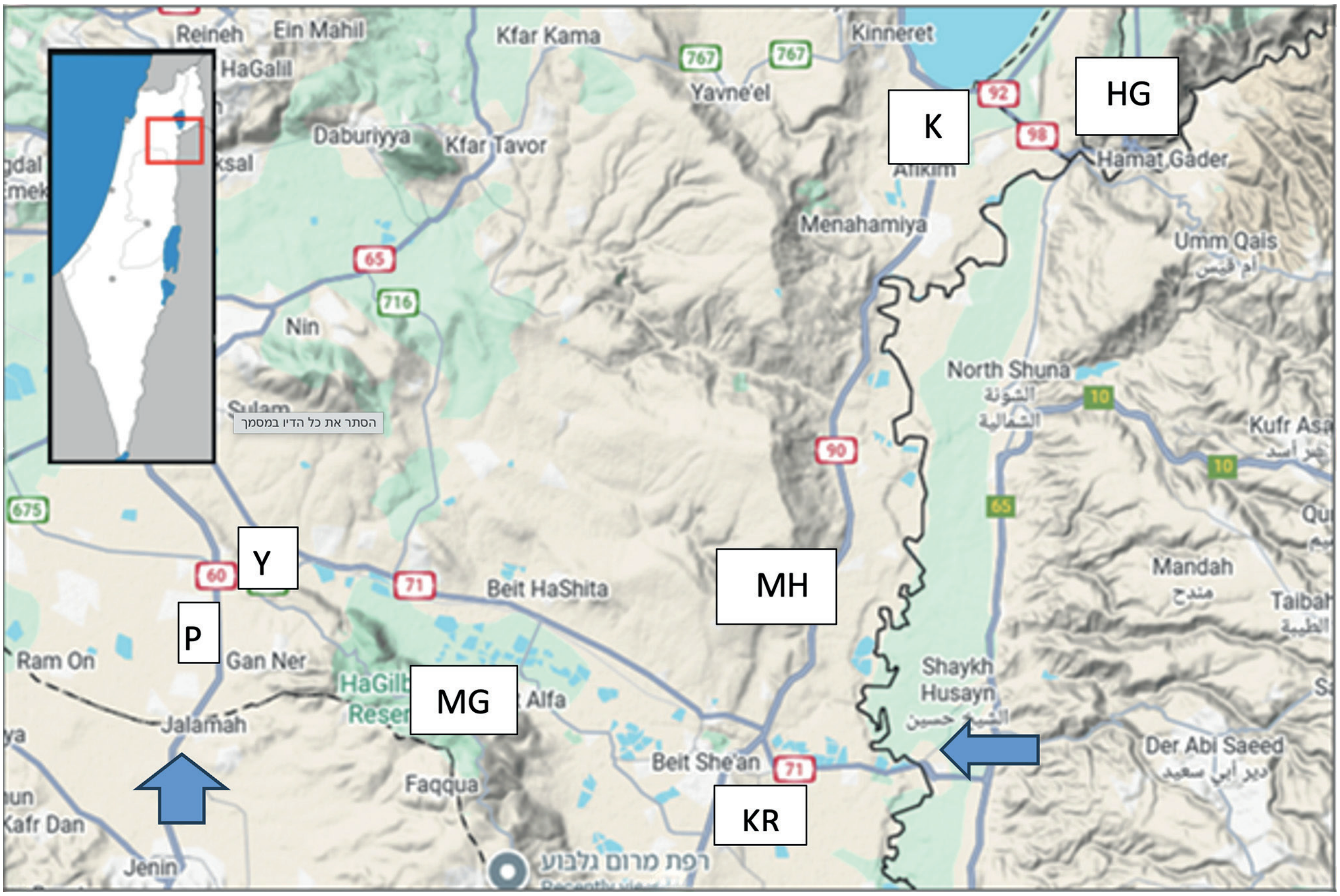

Figure 2. Map of the affected farms in Bet She'an valley between 2019 to 2025. “P” stands for Prazon, site of bTB outbreak at 2025, "Y" stands for KibbutzYizrael, place of infection in 2022, “K” stands for Kinneret 2020 outbreak, “KR” stands for Kfar Ruppin and “MG” stands for Merom Gilboa (Meirav), in which bTB occurred in 2020. “MH” stands for Maoz Haim indicating the bTB event of 2019, “HG” stands for Hamat Gader the source of the zoo outbreak in 2018. The arrows demonstrate the border checkpoints with the West Bank (Palestinian Territories) on the left, and Jordanian border (on the right).

The incidence of bTB in Israeli cattle decreased significantly following a decades-long test-and-cull policy installed by the British Mandatory Veterinary Services and continued following the establishment of the State of Israel. The country was officially “historically free” of bTB since the last diagnosed cases in 2000. Other than a few imported beef cattle diagnosed at slaughter since 2018 and thereafter, there was no evidence of the disease in Israeli livestock until 2019.

In recent years, bTB outbreaks occurs in a specific region in the north of Israel, as described hereby and demonstrated in Figure 2.

In March 2019, an outbreak of tuberculosis caused by *M. bovis* occurred in the Maoz Haim dairy farm in the Beit She'an valley, located 2 km away from the Jordanian border. The outbreak was detected concomitantly in three cows during routine slaughter and a calf during PM investigation *on* the farm. Granulomatous lung lesions were identified in all the animals, from which acid-fast bacteria were demonstrated in two cows. Further laboratory testing confirmed *M. bovis* infection. Subsequent herd-wide tuberculin testing identified 46 reactor animals out of 559 cows and heifers tested, all of which were culled. Repeated test-and-cull in the herd over 2019-2020 failed to prevent new within herd transmissions and to reduce substantially the herd prevalence, ultimately leading to depopulation of the entire herd. Strict quarantine measures were implemented, milk collection was suspended, and no human infections were identified (45).

In September 2020, *M. bovis* infection was confirmed by PCR in a cow from a family-operated dairy farm in Moshav Kinneret, south to Lake Kinneret. First assessment by IGRA

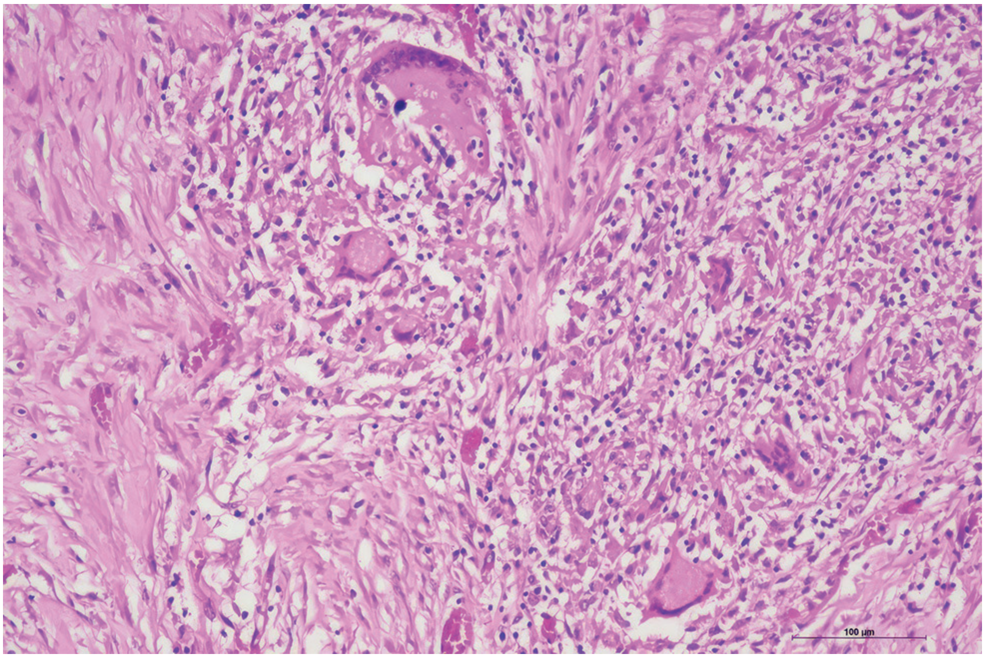

Figure 3. Histopathology of the lung sampled from the first infected heifer in Prazon demonstrating severe granulomatous inflammation with numerous multinucleated giant (Langhans) cells and epithelioid macrophages (H&E stain, 200X magnification). Picture kindly provided by Dr. Nir Edery.

revealed thirteen reactor cows out of 198 tested and an additional 10 suspected ones. This herd too was depopulated, due to increased prevalence in subsequent tests. One month later, another outbreak was identified in a large dual-site dairy operation belonging to Kibbutz Meirav and Kibbutz Kfar Ruppin in the Merom Gilboa region. Among 650 milking cows and 350 heifers, 270 cows and 77 heifers reacted to MTB. This farm is located approximately 15 km from the Prazon outbreak described in the present study, whereas the heifers' extension in Kfar Ruppin is located only 3 km from Maoz Haim, where the first outbreak occurred.

Consequently, for these outbreaks, CTST was performed between January 2021 to April 2021, in dairy farms of nine villages in the Jordan Valley. All bTB tests were negative.

In December 2022, a further case of bovine tuberculosis was detected at the dairy farm of Kibbutz Yizrael, in the Jezreel Valley, following identification of tuberculous lesions in a slaughtered cow, *M. bovis* was confirmed in this cow (by PCR and bacterial isolation). Subsequently, three rounds of herd testing led to the removal of 21 reactor cows out of 853 livestock aged above 6 months of age. The affected farm was located only 1.3 km from the holding described in the present report.

CASE PRESENTATION

On 13 February 2025, a heifer born in April 2023, died suddenly at a dairy farm in Moshav Prazon, located in the Jezreel Valley. The heifer had a history of recurrent pneumonia. *Post-mortem* examination performed by the attending veterinarian revealed disseminated tubercles in the liver, spleen, lungs, peritoneum, and diaphragm. The affected organs were submitted to the Kimron Veterinary Institute for further investigation.

The heifer tested positive by PCR for BTV, Bovine Leukemia Virus (BLV), and members of the *Mycobacterium tuberculosis* complex (MTBC). Acid-fast staining demonstrated more than 10 acid-fast bacilli per smear. Histopathological examination of lung tissue revealed lesions characteristic of tuberculosis (Figure 3). Ultimately, *Mycobacterium bovis*, the causative agent of bovine tuberculosis, was isolated in culture.

The affected holding was a small cooperative dairy farm with an annual milk quota of approximately 700,000 liters. The herd comprised 61 dairy cows, 21 replacement heifers, and 37 calves (fattening and suckling), totaling 119 cattle, distributed among seven compartments. Farm personnel included the owner, his younger son, and one worker. The herd was under the care of a private veterinarian affiliated with a

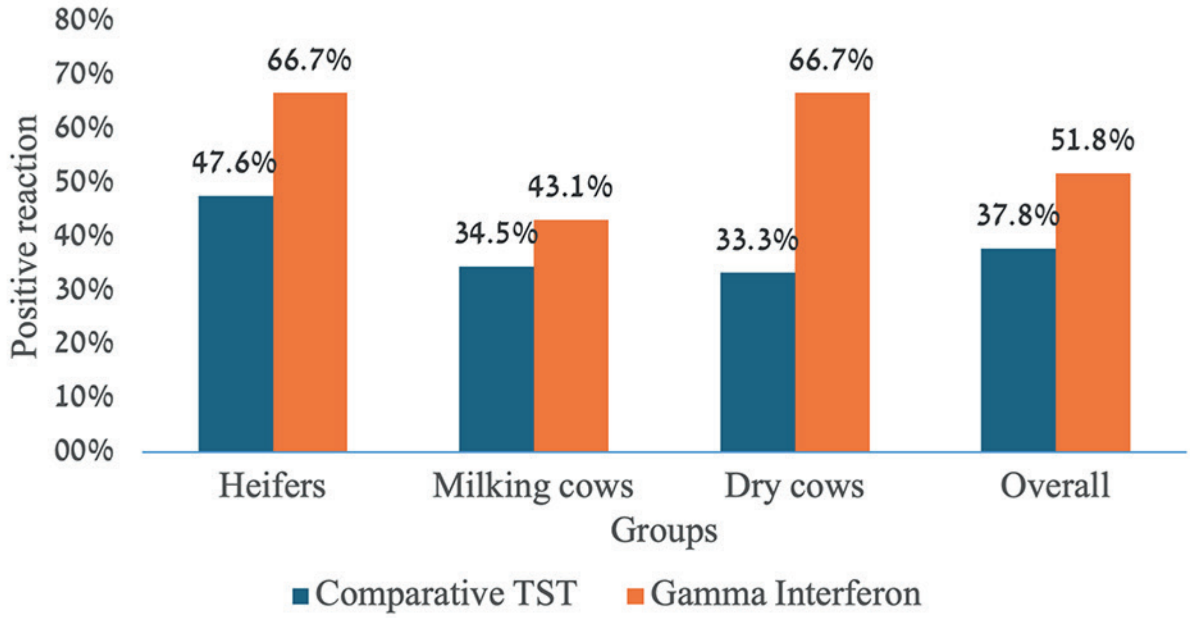

Figure 4. Bovine tuberculosis infection rate among different groups of cattle, at the Prazon outbreak that occurred in February 2025. Cows and Heifers were tested using CTST and Gamma interferon methods.

cooperative for clinical veterinary services owned by farmers in Israel, who visited the farm twice weekly on fixed days.

In December 2024, the entire herd was vaccinated against foot-and-mouth disease (mandatory vaccine). Other vaccinations were not documented. The farm did not maintain computerized records, and no documentation of previous tuberculin testing was found. Total mixed ration for lactating and dry cows was delivered daily from the feed center of Tel Yosef. Grower ration was purchased three times weekly from a feed center in Sde Ilan, and concentrate feed was also supplied by the same feed center. Calf feeding management included the use of unpasteurized colostrum, and waste milk/high-somatic-cell milk was fed to calves. Milk was marketed to the Tnuva dairy plant in Tel Yosef four days per week.

Additional personnel entering the farm on a regular basis included an artificial insemination technician (on demand) and a hoof trimmer who visited annually in the month of September. Manure removal was performed independently by the farm owner. Regarding animal movements, during 2024 the farm introduced five cows and six pregnant heifers from a dairy farm in Moshav Yavne'el. The source farm had subsequently undergone tuberculin testing and was found to be negative. A review of animal movements out of the farm over the previous three years showed that since 2022 no animals had been transferred for breeding purposes. Eleven calves and fifteen cows were sent to slaughterhouses, all without notable tuberculosis-related findings at *post-mortem* inspection.

Following delivery of the positive diagnostic results and in accordance with the national guideline "Tuberculosis Disease – Performance of the Tuberculin Test" (guideline number 03-01.13, updated 21 September 2016) (46), the comparative tuberculin skin test was conducted in March 2025 on lactating cows, dry cows, and replacement heifers aged six months and older. Concurrently, blood samples were collected for interferon-gamma testing, which was performed at the Bacteriology Laboratory of the Kimron Veterinary Institute.

Within the affected farm, comparative tuberculin testing identified 30 reactor cattle out of 81 tested (37%). Interferon-gamma testing detected 42 positive animals out of 79 with valid results (53%). Overall, 45 out of 81 cattle (56%) were positive in at least one of the two diagnostic tests. The description of tests results is expounded in **Figure 4**.

In light of the high within-herd prevalence and after consideration of additional factors, such as management, farm infrastructure and economic viability, a decision was made by the Israeli Veterinary Services, to depopulate the

Table 1. Pairwise Cohen's kappa between tests performed in the Prazon bTB outbreak. A Positive "Reactor Status" was given to an animal positive in either CTST4 or IGRA. In bold, statistically (*P*<0.05) kappa values.

	CTST4	CTST2	IGRA	LESIONS	PCR	Reactor Status
CTST4	-					
CTST2	**0.684**	-				
IGRA	**0.602**	**0.619**	-			
LESIONS	**0.268**	**0.088**	**0.325**	-		
PCR	**0.362**	**0.277**	0.250	**0.102**	-	
Reactor Status	**0.640**	**0.652**	**0.975**	**0.316**	0.240	-

Cohen's Kappa test agreement:	Almost Perfect	Substantial	Fair	Slight

entire herd. Ninety-nine animals were sent for slaughter at the Yarka abattoir, while the remaining 23 animals, deemed unfit for slaughter, were euthanized at the farm. At slaughter, tuberculosis-suspect lesions were identified in lymph nodes and/or organs of 49 animals (51%). In several animals, lesions were detected in more than one lymph node or organ. Twenty one percent of the sampled tissues were positive for the *Mycobacterium tuberculosis* complex (MTBC) by direct PCR, and the bacterium was successfully isolated in culture in a few samples tested, confirming the infection. The percentage of animals with lesions by site was as follows: 35% mediastinal lymph node (LN), 33% liver, 25% thoracic LN, 23% retropharyngeal LN, 17% tracheal LN, 10% lungs, 10% submandibular LN, 4% pre-scapular LN, and 4% parotid LN.

Because an almost complete set of diagnostic data was available, it was possible to investigate the different tests performances in this outbreak setting. Firstly, an overall agreement was calculated using the Fleiss' kappa statistic. For this we used CTST at two different cut-offs, the standard 4 mm cut-off (CTST4) and the "severe" 2 mm interpretation (CTST2), IGRA, and the presence of TB-associated lesions at slaughter. A moderate (kappa=0.42) but highly statistically significant (P<0.0001, n=77) association between all tests was found. We then performed a pairwise comparison of tests using the Cohen's kappa statistic, including the "Reactor Status" as animals positive in either CTST4 or IGRA (Table 1). Notably, there was substantial agreement between both *ante-mortem* tests, CTST and IGRA, but only fair agreement between any of these two tests, or both combined, and the presence of bTB lesions *post-mortem*. Accordingly, the Odds Ratio for finding bTB lesions was 3.87 for IGRA and 3.33 for CTST4 (*P<0.05*). Of note was also the overall low agreement between PCR and the other tests, especially "Reactor Status". This was in accordance with the paucibacillary nature of closed and limited bTB lesions, and illustrates the limitations of using PCR (or culture) to confirm infection status for compensation purposes.

In this particular setting, CTST, IGRA and both combined showed a Sensitivity of 50%, 68% and 70%, and Specificity of 77%, 64% and 62%, respectively, when using the presence of Lesions *post-mortem* as the "gold standard".

In accordance with the law, the farm owner received compensation for the animals slaughtered or euthanized. Prior to restocking the farm, the owner was provided with a detailed list of required cleaning and disinfection procedures, including a mandatory waiting period for repopulation.

Comparative tuberculin testing was performed in all dairy farms within a maximum radius of 3 km from the affected holding. A sample of approximately 175 cows and heifers older than six months was tested in a total of 14 dairy farms (Figure 5). Sample size was designed to detect at least one infected animal in a herd with a minimum prevalence of 5%, considering an estimated Sensitivity of 50-80% and Specificity of 90% for the CTST. All farms tested negative. In one farm, three reactor cows were found, none showing TB lesions at slaughter, and the farm was therefore regarded as TB free.

DISCUSSION

Sources of Infection

The epidemiological investigation did not identify a definitive source for the introduction of bovine tuberculosis (bTB) into the Prazon dairy farm. Introduction through purchased

Figure 5. The 14 dairy farms that were tested within a maximum radius of 3 km from the affected holding. Green spot indicates negative farms, blue spot is a positive farm. The distance between the affected farms, in Yizrael and Prazon, as indicated by the arrow is 1.3 km.

animals was not supported, as the source farm tested negative using the comparative tuberculin skin test (CTST). However, this route cannot be completely excluded, since mandatory pre-movement testing in Israel does not include CTST, and routine national surveillance using CTST has been discontinued since 2015.

Despite Israel's previous designation as "historically free" of bTB prior to its re-emergence in 2019, several potential infection sources have existed. Molecular evidence of bTB west of the Jordan River was reported in 2013 in a study conducted in the West Bank (Palestinian Territories). In that study, *Mycobacterium tuberculosis* complex (MTBC) DNA was detected in approximately 3% of sampled livestock, and *Mycobacterium bovis* was confirmed in at least one case using molecular techniques. Detection of *M. bovis* DNA in both tissues and raw milk samples suggested that apparently healthy animals may act as carriers and highlighted the zoonotic risk associated with raw milk consumption (47).

Additional regional data indicate the continued presence of bTB in neighboring countries, including Jordan, as reported through WOAH surveillance systems. Earlier regional reviews also documented bTB in several Middle Eastern countries, although quantitative prevalence data remained limited (48). These findings suggest that bTB persists in the region surrounding Israel and may involve both livestock and wildlife reservoirs.

A significant outbreak between 2018 and 2019 in eight zoological collections in northern Israel further supports this assumption. The outbreak originated from a deer-holding facility in Hamat Gader, near the borders with Jordan and Syria. A high proportion of spotted deer (*Axis axis*) tested positive, and multiple additional species were affected, including Nubian ibex (*Capra nubiana*), mountain gazelle (*Gazella gazella*), tufted capuchin monkey (*Sapajus apella*). Although the outbreak was traced to a single captive population, the initial source of infection remained undetermined, and spillover from free-ranging wildlife was considered a plausible route.

Wildlife reservoirs play a critical role in the epidemiology of bTB, although the key species vary geographically. Examples include possums in New Zealand, badgers in the United Kingdom and Ireland, white-tailed deer and bison in North America, and African buffalo in Africa (49).

In Israel, wildlife surveillance is conducted within the Israel Wildlife Disease Surveillance (IWDS) program, based primarily on serological testing (50). The general TB incidence in wildlife throughout the country is demonstrated in Figure 6, while the findings in Ha'amakim Valley are presented in Figure 7. Between 2021 and 2025, 39 positive cases were identified, mainly clustered in the Beit She'an and Harod Valley regions, areas where recent cattle outbreaks have also been reported (Fig.6 and Fig.7). Affected species included the golden jackal (*Canis aureus*), Indian crested porcupine (*Hystrix indica*), wild boar (*Sus scrofa*), and Persian fallow deer (*Dama mesopotamica*). Among these, the golden jackal is particularly relevant due to its frequent proximity to dairy farms. Jackals are attracted to farm environments, especially calving areas, where they seek food and water, and may contribute to indirect transmission by contaminating shared resources such as water troughs.

Taken together the correspondence between the positive results in wildlife and in cattle farms, suggest that this region may represent a hotspot for *Mycobacterium bovis* circulation. The combined effects of wildlife movement and livestock trade likely facilitate pathogen dissemination. Nevertheless, the precise role of wildlife in disease transmission remains unclear and warrants further investigation, particularly through molecular typing and comparison of strains from wildlife and cattle.

Disease Spread Within the Herd

The relatively high prevalence observed among young replacement heifers (66.7% by IFN-γ assay and 47.6% by CTST) may be explained due to several factors. Firstly, the heifer housing is physically separated from adult cows and located adjacent to open fields, potentially increasing exposure to wildlife. Secondly, feeding practices, specifically the use of unpasteurized colostrum and waste milk, may have contributed significantly to within-herd transmission.

Given the typically slow progression of tuberculosis, with granuloma formation developing over months to years, the high infection rate suggests prolonged and ongoing exposure within the herd.

Infection in Young Animals

Age is a well-recognized risk factor for bTB. While cumulative exposure increases with age (39), infection may occur early in life, with clinical signs and detectable lesions appearing only later on (51). Additionally, older animals may exhibit reduced reactivity to tuberculin testing due to diminished immune responsiveness (52).

In the present case, a high proportion of young animals were affected: 67% of the heifers tested positive, and 42% already exhibited pathological lesions. Similar lesions were observed in a subset of fattening calves. These findings indicate early exposure and suggest a persistent infection source within the farm environment.

Importance of *Post-mortem* Inspection

The actions of the attending veterinarian were critical for early detection of the outbreak. The decision to perform a post-mortem examination and submit samples for laboratory analysis enabled a timely diagnosis. This was not an obvious step, given the low incidence of bTB in Israel. However, awareness of recent cases in nearby locations contributed to appropriate clinical suspicion and decision-making, ultimately improving the outbreak management.

Economic Consequences

Bovine tuberculosis can have substantial economic impacts on the dairy industry. As a chronic disease, it is associated with reduced productivity, including decreased milk yield, weight loss, and impaired reproduction, as well as compromised animal welfare (53). Beyond direct production losses, infected farms face also indirect economic consequences, such as trade restrictions, loss of genetic potential, and need of replacement calves (54). In Israel, control measures, including testing and culling, are fully funded by the State. Farmers are compensated for direct losses only.

Management and Surveillance Challenges

Treatment of bTB in cattle is impracticable due to cost, limited efficacy, risk of antimicrobial resistance, milk discard, environmental contamination, and zoonotic concerns (55).

Vaccination is not implemented either, primarily because the available BCG vaccine interferes with the current standard diagnostic tests, which are based on cell-mediated immune responses (56). Although vaccination has been

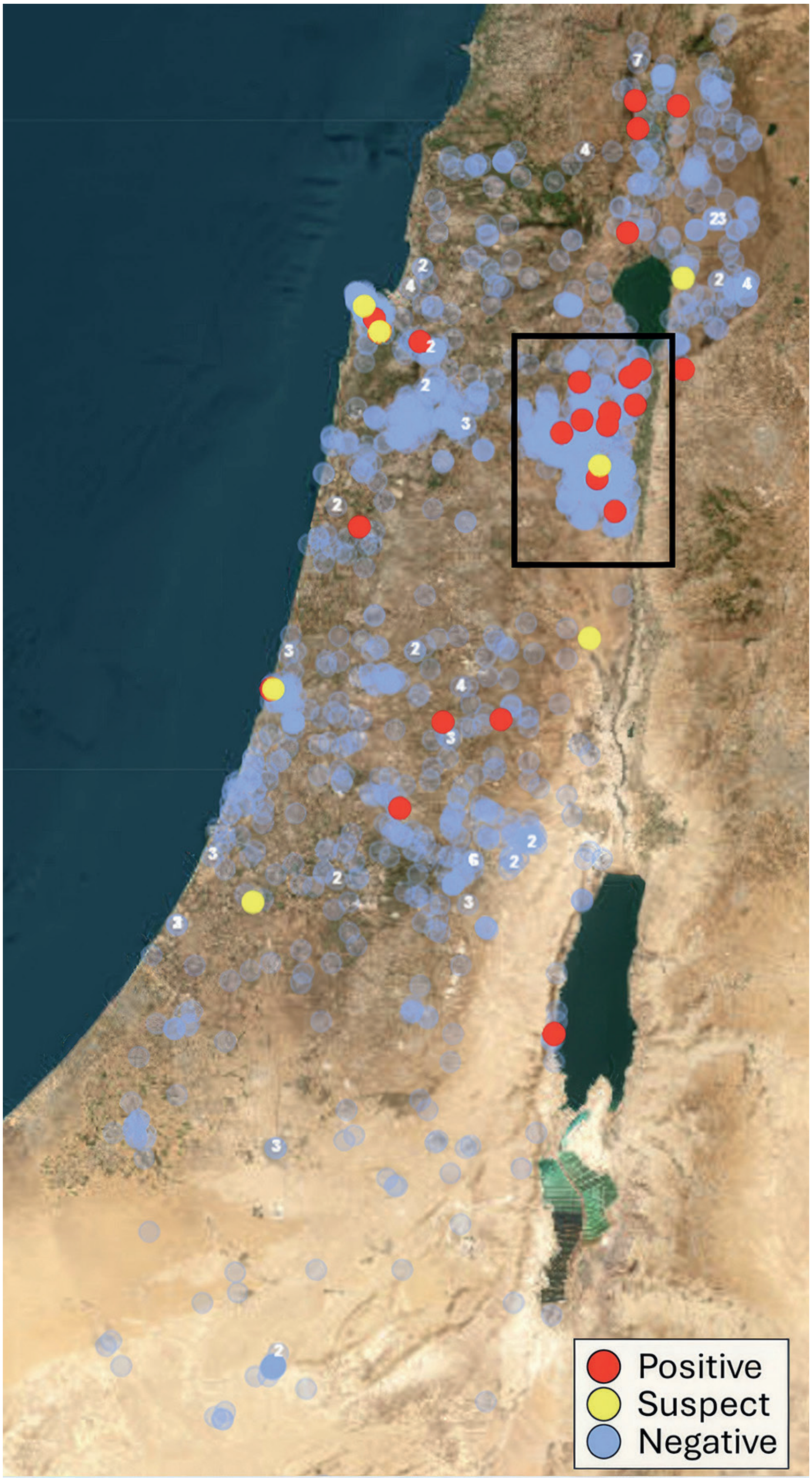

Figure 6. Map of Tuberculosis serological surveillance in wildlife in Israel, depicting samples tested in the years 2021-2025. A total of 2,364 samples were analyzed using ELISA, from which 1,837 were negative, 39 were positive and 8 were suspected for *M. tuberculosis*-complex exposure. The cluster of positive samples in wild animals in the Ha'amakim Valley is clearly visible. As marked in the black rectangle.

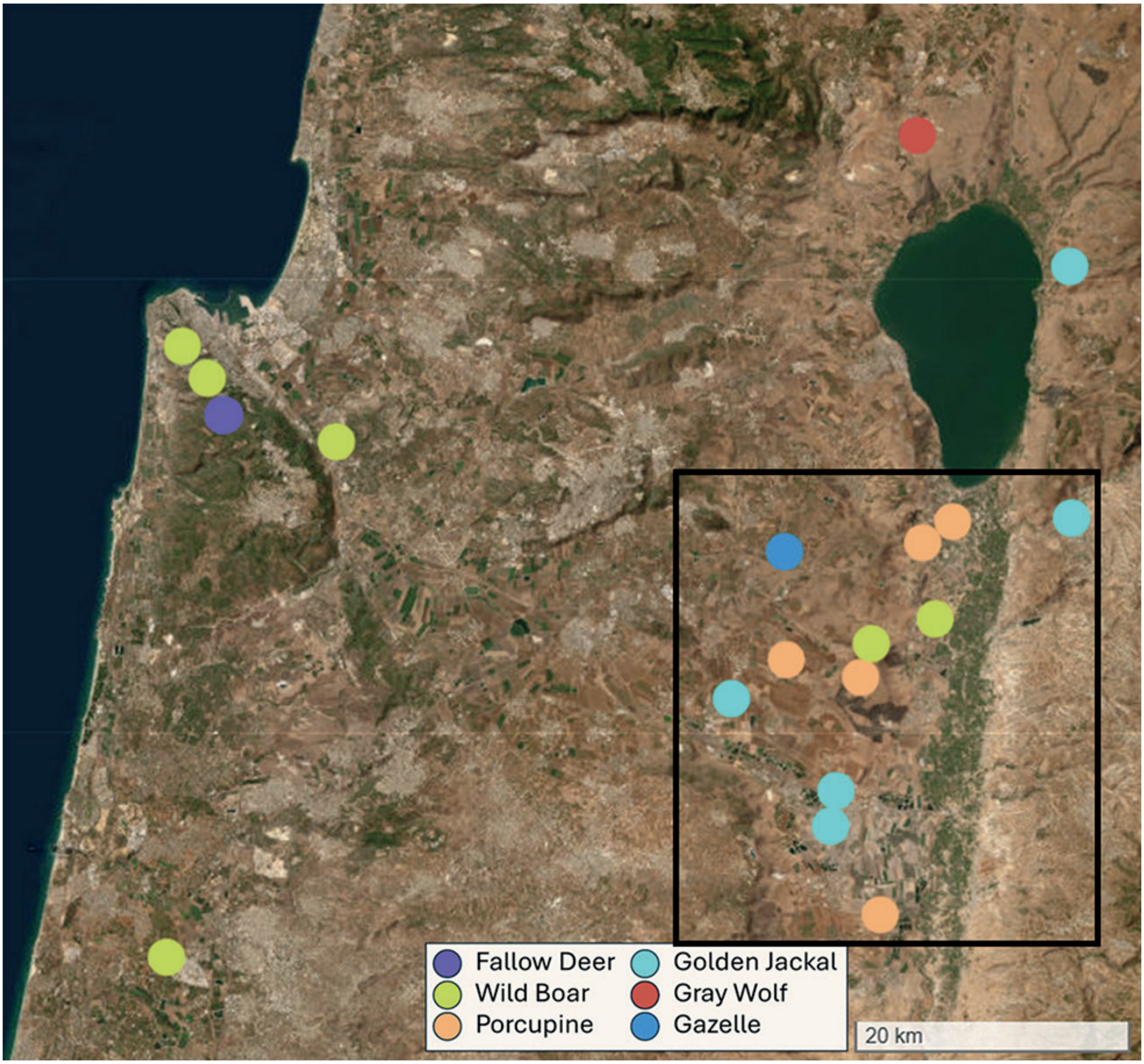

Figure 7. Serological positive wild animals in Israel. Between 2021 and 2025, three golden jackals, five porcupines, two wild boars and one gazelle were found positive specifically in the Ha'amakim region, as marked in the black rectangle.

proposed as a long-term control strategy in countries with established wildlife reservoirs, such as the United Kingdom and New Zealand (57), its effectiveness in cattle remains variable, and further research is required (58).

Surveillance and control strategies vary internationally. In the United States, where bovine tuberculosis was nearly eradicated and herd prevalence remains very low, control measures included routine herd testing, targeted use of IFN-γ assay, slaughterhouse surveillance, and strict regulatory oversight. Wildlife reservoirs, particularly deer, continue to pose a challenge in specific regions (59, 60). Within the European Union, surveillance approaches differ according to disease status. Countries classified as bTB-free rely mainly on passive surveillance at slaughter, whereas endemic countries implement regular mandatory testing of cattle herds (61).

In Israel, routine tuberculin testing was discontinued in 2015 due to the low incidence of disease observed since the 1990s and the absence of diagnosed bovine tuberculosis (bTB) cases since 2000. Since then, surveillance has relied primarily on routine abattoir inspections and occasional on farm *post-mortem* examinations. However, dairy cows sold to farmers in the Palestinian Territories are effectively lost to follow-up, as *post-mortem* data are generally not accessible to the Israeli Veterinary Services. This creates a significant gap in the surveillance system, further complicated by the fact that animals selected for such sales are often older, lower-value cows, which are inherently at higher risk of chronic infections such as bTB.

Furthermore, reliance on slaughterhouse surveillance alone is limited by uneven submission rates, as some farms send few or no animals for slaughter directly. This reduces the effectiveness of passive surveillance and may delay detection of infection. For instance, a regional survey conducted in the Ha'amakim district in 2024 revealed that, of 142 dairy farms, five did not send any cattle to slaughter, and approximately 20 farms sent only negligible numbers annually. These findings highlight the limitations of relying primarily on *post-mortem* inspection at slaughterhouses as the main surveillance tool.

In general, Israel maintains a highly regulated cattle industry, with individual animal identification, controlled movement, and centralized slaughterhouse inspection. Nevertheless, the absence of active surveillance and pre-movement testing in "hot-spot" regions represents a weakness of the current system.

Since family farms send less cows to slaughter houses, it might be warranted to request CTST every 3-5 years, from farms which don't meet a standard of PM examinations at slaughter, in order to maintain a TB-free status, especially in the area of the Beit She'an valley.

Countries with regional differences in the incidence of bTB may opt to adopt different local control policies accordingly. In Italy, for example, where most bovine tuberculosis (bTB) outbreaks have been reported in the Toscana region, surveillance programs in bTB-free territories include testing of all bovine animals older than six weeks, in a percentage of herds specific for each territory. Introduction of cattle into an bTB-free farms is allowed from bTB-free herds only. Likewise, fattening farms must be populated exclusively with animals from bTB-free sources (62). In Israel, in contrast, when lesions are detected during routine slaughter, all animals over six months of age in the originating farm are subsequently tested using either the TST and the interferon-gamma assay to maximize the identification of reactors. Epidemiological trace-back of animals originating from the suspected herd is established, and the respective farms are screened by TST. The requirement for bTB freedom for fattening farms is not currently implemented in Israel. The whole chain of inspection and diagnosis is highly centralized: the bulk of cattle is culled in the only five licensed abattoirs nationwide, carcasses are inspected by veterinarians under the professional auspices of the Veterinary Services, and suspicious lesions are referred exclusively to the Kimron Veterinary Institute for further thorough laboratory investigation. In countries with well-established surveillance and control programs, bTB incidence is generally kept low and transmission remains limited (63). To remain optimal, surveillance and control should be periodically updated to align with the current epidemiological status.

CONCLUSIONS

The outbreak occurred on a family-owned dairy farm comprising a total of 119 cows and heifers. It was first identified in a young heifer that died on the farm following a clinical presentation consistent with pneumonia. Consequently, the entire herd was tested using both the tuberculin skin test and the interferon-gamma assay. Of the animals tested, 38% were positive by the tuberculin test, while 52% were positive by the interferon-gamma assay. Furthermore, post-mortem examination at slaughter revealed typical tuberculosis lesions in 44% of the cattle.

The high prevalence observed among young replacement heifers suggested that feeding practices likely played a significant role in within-herd transmission. Specifically, the use of unpasteurized colostrum and waste milk for feeding suckling calves was identified as a major risk factor. Accordingly, pasteurization of colostrum and waste milk was strongly recommended, not only to reduce the risk of bTB transmission but also to protect calves from other infectious diseases.

A second important risk factor identified was the accessibility of wildlife to the farm environment. Unlike regions where one or two wildlife species typically serve as reservoirs, in the Ha'amakim Valley it appears that multiple species, including jackals, porcupines, wild boar, and Persian fallow deer, may act as reservoirs. The high density and close proximity of wildlife and livestock in this region likely facilitate disease transmission, particularly when biosecurity measures are not strictly maintained.

Notably, four previous bTB outbreaks reported over the past six years in Maoz Hayim, Yizrael, Kinneret, and Merom Gilboa are all located within a radius of approximately 30 km from Prazon. Kinneret is situated about 30 km away, Merom Gilboa 15 km, and Yizrael only 1.3 km from the affected farm. The Ha'amakim Valley is characterized by intensive livestock farming and frequent animal movement. For example, the Gilboa checkpoint to the West Bank is located approximately 4 km from Prazon, while the border crossing to Jordan is about 23 km away. Route 60, a major transportation corridor for cattle movement into the West Bank, passes in close proximity to the affected farm. In addition, epidemiological evidence from wildlife, captive animals, and livestock suggested a southward spread of bTB from the area south of Lake Kinneret toward the Jordan Valley. The recurrence of cases indicated that the pathogen is well established in the Ha'amakim Valley region, and further outbreaks are likely.

Currently, tuberculosis surveillance in Israel relies predominantly on detection at slaughterhouses. However, family farms often send relatively few animals to slaughter, limiting the effectiveness of this approach. Therefore, periodic herd testing using the comparative tuberculin skin test every 3-5 years may be warranted for farms that do not meet a minimum threshold of *post-mortem* surveillance, particularly in high-risk areas such as the Ha'amakim Valley. In addition, on-farm *post-mortem* examinations and submission of samples to diagnostic laboratories are critical for improving case detection.

It is also important to note that some farms transfer animals to areas under the Palestinian Authority rather than to Israeli slaughterhouses, thereby bypassing routine abattoir surveillance. This practice creates a gap in the surveillance system and may facilitate undetected disease spread.

To reduce the risk of introduction and transmission of bTB in dairy farms, strict biosecurity measures should be implemented. These include effective fencing to limit wildlife access and the use of pasteurized milk or milk replacers for calf feeding. Furthermore, systematic surveillance of tuberculosis in wildlife should be expanded and institutionalized, i.e. transitioning from convenience sampling to statistically representative and systematic sampling, particularly in the Ha'amakim Valley. Most importantly, surveillance in wildlife must incorporate tissue sampling for actual pathogen detection.

Finally, continuous risk communication with farmers regarding identified risk factors is essential to support effective disease prevention and early detection.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENT

The authors wish to express their sincere appreciation for the dedicated efforts of the "Ha'amakim veterinary district" staff.

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