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# Evaluation and application of a milk antibody ELISA for assessing the prevalence and incidence of bovine tuberculosis in dairy herds in Hubei Province, China

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## Abstract

Bovine tuberculosis (bTB) is a chronic zoonotic disease that is endemic in China. Current *in-vitro* tests for bTB are mainly based on blood assays. Collection of samples results in some stress to the sampled cattle and associated economic losses for the herd owner. This study was designed to investigate the relationship between milk and serum antibody tests for bTB in dairy cows using 85 cows with milk and corresponding blood samples. Totally 4,395 milk samples were used to assesse the apparent (test) prevalence and incidence of bTB using the milk antibody ELISA. The association between levels of bTB milk antibody and milk quality was also evaluated. Milk and serum antibody tests showed a good correlation with a 87.5% (95% CI: 61.7%, 98.4) positive agreement and 98.7% (95% CI: 95.4, 99.8) negative agreement. The animal level lactoprevalence ranged from 0.3% (95% CI: 0, 1.2) to 33.3% (95% CI: 26.6, 40.6) in different farms and the incidence rate ranged from 0 head/cow-month (95% CI: 0, 0.02) to 0.04 head/cow-month (95% CI: 0.02, 0.07). Twenty percent of sampled farms met the criteria for bTB control in China. The prevalence on large-scale farms was lower ($p < 0.001$) than on small farms. The bTB milk antibody levels had a negative correlation with milk yield and a positive correlation with somatic cell count (SCC), milk protein percentage (MPP) and percentage of total solids (TS). According to this research, milk ELISA could be used as a supplement of blood samples to assist in the surveillance for bTB and for alerting control and eradication of bTB.

**Keywords** Bovine Tuberculosis, Milk antibody, Prevalence, Incidence rate, Milk quality

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## Introduction

Bovine tuberculosis (bTB) is a chronic zoonotic disease resulting in a significant economic loss to the livestock industry and potentially having an important impact on human health (Olea-Popelka et al. 2017). It is mainly caused by *Mycobacterium bovis* (*M. bovis*), and can be transmitted to humans through close contact with infected animals or consumption of unpasteurized dairy products (Katale et al. 2012; Romha et al. 2018). bTB leads to reduced productivity, trade and movement restrictions on livestock and their products (Sibhat et al. 2017). Due to its severe impact on both human health and the livestock industry, the China Ministry of Agriculture and Rural Affairs classified bTB of dairy cattle as one of the 16 infectious diseases to be controlled and eliminated in its Medium and Long-Term National Plan for Prevention and Control of Animal Diseases (2012–2020) (http://www.moa.gov.cn/xw/zwdt/201205/t20120530_2678977.htm). However, after eight years of implementation, bTB has been only successfully eliminated in a few breeding farms of China. A lack of awareness of the disease's severity by producers due to the nature of chronic and latent infections, and the complexity or ambiguity of diagnostic tests have hindered the control and elimination of bTB in the country. A 7-year investigation indicated that the true animal level prevalence of bTB in China could be as high as 59.5% (95% CI: 48.2, 70.0) in some farms (Chen et al. 2018).

The current control strategy for bTB in China is "test-and-slaughter", together with surveillance and movement restrictions. The intradermal tuberculin skin test and IFN-γ test are the two recommended bTB tests by the national standards in China (GB/T 18,645–2020, GB/T 32,945–2016), and a serum antibody test for bTB is referred to in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals edited by World Organisation of Animal Health (WOAH) (https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.13_Mammalian_tuberculosis.pdf). However, these tests are time- and labor-consuming, and unsuitable to quickly screen large numbers of animals (Cho et al. 2015).

Milk-based tests have been developed for the survey and surveillance of other diseases including bovine brucellosis (Wang et al. 2020), *Mycoplasma bovis* disease (Parker et al. 2017), paratuberculosis (Bauman et al. 2019) and bovine leukosis (Evermann et al. 2019). Collection and testing of milk samples offers the advantages of less impact on dairy cows and increases the likelihood of producers cooperating for bTB surveillance. In addition, milk samples of each lactating cow are already collected monthly and tested as part of the China National Dairy Herd Improvement (DHI) Program for assessing breeding suitability and milk quality and for the early detection of mastitis using CombiFoss FT + milk composition and a somatic cell analyzer. Therefore it would be ideal to combine bTB surveillance with the existing DHI Program if a milk test were available considering the low cost, simplicity and noninvasiveness of sample collection.

This study was undertaken to assess a milk test for bTB by comparing the agreement between milk and serum antibody tests based on the MPB70/ MPB83/CFP-10/ ESAT6 fusion protein of *M. bovis*. The milk test was then used to determine the prevalence and incidence rate of bTB and to evaluate the association between lactoprevalence and milk quality in 15 commercial dairy farms in Hubei Province of China.

## Results

### Comparison between milk and serum tests

Of the 85 animals with milk and corresponding blood samples tested for bTB antibodies, seven animals (8.2%, 95% CI: 3.4, 16.2) were tested positive to serum ELISA (reference test), and all of them (100%, 95% CI: 59.0, 100.0) were positive to milk ELISA. Of the 78 negative animals to serum ELISA, 76 (97.4%, 95% CI: 91.0, 99.7) were also negative to milk ELISA (Table 1). The overall agreement between milk and serum antibody ELISA was 97.7% (95% CI: 94.1, 99.4), with an 87.5% (95% CI: 61.7, 98.4) positive agreement and 98.7% (95% CI: 95.4, 99.8) negative agreement. The kappa value was 0.862 (95% CI: 0.7, 1.0) ($p < 0.001$), indicating that the results of milk and serum ELISA had a good level of agreement (Thrusfield 2018).

### Apparent (test) prevalence

Overall 7.9% (347/4395) milk samples were test positive on the ELISA. The highest lactoprevalence was 41% (25/61, 95% CI: 28.6, 54.3) in farm O in Jan 2018, followed by 35.9% (33/92, 95% CI: 26.1, 46.5) in farm M in March 2019. The lowest lactoprevalence was in farm A (0%, 0/197, 95% CI: 0, 1.9) in Jan 2018. The overall lactoprevalence in different farms varied from 0.3% (2/590, 95% CI: 0, 1.2) to 33.3% (62/186, 95% CI: 26.6, 40.6) (Table 2). Only three farms (A, B, K) had a lactoprevalence < 3%, meeting the criteria for controlling bTB in China (http://www.moa.gov.cn/nybgb/2017/dqq/201712/t20171230_6133930.htm, animal level prevalence < 3% for the past two consecutive years).

When sample collection time was analysed, both Mar 2019 (OR = 1.7, 95% CI: 1.2, 2.4, $p = 0.002$) and June 2019 (OR = 2.2, 95% CI: 1.6, 3.1, $p < 0.001$) had significantly higher lactoprevalences compared with Apr 2018 (Table 3). Cows sampled in 2019 also had a significantly higher lactoprevalence compared with those sampled in 2018 (OR = 0.6, 95% CI: 0.5, 0.7, $p < 0.0001$).

**Table 1** Cross-classification of milk and serum ELISA results for bTB on three farms in Hubei Province, China

| | | Serum test | | Total |
|---|---|---|---|---|
| | | + | - | |
| **Milk test** | + | 7 | 2 | 9 |
| | - | 0 | 76 | 76 |
| **Total** | | 7 | 78 | 85 |

When size of herds was assessed, the total animal level lactoprevalence in large-scale farms (4.3%, 95% CI: 3.4, 5.3) was lower ($p < 0.001$) than that in small farms (10.6%, 95% CI: 9.4, 11.9) (Table 3).

### Incidence rate

The overall test incidence rate was calculated to be 0.01 head/cow-month (95% CI: 0.008, 0.013) for 14 dairy farms (farm J had no samples that met the criterion for calculating) (Table 4). Incidence rate of different farms ranged from 0 head/cow-month (95% CI: 0, 0.02) to 0.04 head/cow-month (95% CI: 0.02, 0.07). There was no significant difference ($p = 0.34$) in the incidence rate for lactoconversion to bTB between large farms (A, B, C and D) and small farms (E-O).

### Influence of bTB on milk quality

The influence of bTB antibody lactopositivity on milk quality was then analyzed including milk yield, fat content, milk protein percentage (MPP), lactose content, percentage of total solids (TS), somatic cell counts (SCC, × 1,000 cell/mL) and urea nitrogen content. Herds with a high prevalence (≥ 3%) had a significantly lower milk yield ($p < 0.01$), but a higher MPP ($p < 0.01$), TS ($p < 0.05$) and SCC ($p < 0.01$) than herds with a low prevalence (< 3%). There were no significant differences

**Table 2** Animal level lactoprevalence to bTB based on an ELISA[a] in 15 farms in Hubei Province, China

| Farm | Prevalence (95% CI[b]) | | | | | |
|---|---|---|---|---|---|---|
| | **2018. 01** | **2018. 04** | **2018. 07** | **2019. 03** | **2019. 06** | **Total** |
| A | 0.0% (0, 1.9) | 0.0% (0.0, 2.3) | 0.0% (0,3.8) | 1.4% (0, 7.6) | 1.4% (0, 7.6) | 0.3% (0, 1.2) |
| B | 0.0% (0, 3.2) | 0.0% (0.0, 3.5) | 1.6% (0, 8.8) | 1.5% (0.2, 5.4) | - | 0.7% (0.2, 2.1) |
| C | - | 0.6% (0.0, 3.6) | 0.9% (0, 4.8) | 7.0% (2.3, 15.7) | 8.5% (3.2, 17.5) | 3.2% (1.7, 5.4) |
| D | - | 7.7% (4.1, 13.1) | 18.3% (13, 24.8) | | 12.8% (7.7, 19.4) | 13.2% (10.3, 16.6) |
| E | 2.4% (0.5, 6.8) | 1.9% (0.2, 6.7) | 20.3% (11.8, 31.2) | 13.3% (7.5, 21.4) | 19.4% (8.2, 36) | 9.2% (6.7, 12.3) |
| F | 19.6% (12.2, 28.9) | 20.0% (11.6, 30.8) | - | - | - | 19.8% (14.1, 26.5) |
| G | 10.1% (5.0, 17.8) | 9.0% (3.7, 17.6) | - | - | - | 9.6% (5.7, 14.9) |
| H | 0.0% (0.8, 7.0) | 11.6% (6.9, 18.0) | - | - | - | 7.5% (4.4. 11.8) |
| I | 8.6% (4.5, 14.6) | 4.3% (1.2, 10.8) | - | - | - | 6.9% (4.0, 11) |
| J | 7.6% (2.1, 18.2) | 13.7% (5.7,26.3) | | | | 10.6% (5.4,18.1) |
| K | 0.7% (0, 4) | - | 0.0% (0, 3.7) | 0.0% (0, 5.1) | 0.0% (0, 5.1) | 0.3% (0, 1.5) |
| L | - | 3.9% (0.8, 11.1) | - | 0.0% (0, 5.6) | | 2.1% (0.4, 6.1) |
| M | - | - | - | 35.9% (26.1, 46.5) | 30.9% (21.7, 41.2) | 33.3% (26.6, 40.6) |
| N | 6.6% (2.5, 13.8) | 7.3% (2.7, 15.2) | - | 9.5% (3.6, 19.6) | 11.1% (4.6, 21.6) | 8.4% (5.5, 12.1) |
| O | 41.0% (28.6, 54.3) | 11.1% (3.7, 24.1) | - | 20.5% (9.8, 35.3) | - | 26.0% (19.2, 33.8) |
| Total | 6.7% (5.3, 8.3) | 6.0% (4.8, 7.4) | 8.0% (6.0, 10.5) | 9.9% (7.8, 12.3) | 12.4% (9.8, 15.5) | 7.9% (7.1, 8.7) |

[a] ELISA antibody detection kits were purchased from Wuhan Keqian Biology Co., Ltd

[b] 95% confidence intervals (CI) were calculated for each parameter using the method of Ross (Ross 2003)

**Table 3** Prevalence of bTB at different times and in different sized farms

| | | Number of positive samples | Total | Prevalence (%) (95% CI) | OR (95% CI) | *p* value* |
|---|---|---|---|---|---|---|
| Month | Jan-2018 | 80 | 1196 | 6.7 (5.3, 8.3) | 1.1 (0.8, 1.6) | 0.47 |
| | Apr-2018 | 79 | 1319 | 6.0 (4.8, 7.4) | 1.0 | |
| | Jul-2018 | 50 | 622 | 8.0 (6.0, 10.5) | 1.4 (0.9, 2.0) | 0.09 |
| | Mar-2019 | 70 | 711 | 9.8 (7.8, 12.3) | 1.7 (1.2, 2.4) | 0.002 |
| | Jun-2019 | 68 | 547 | 12.4 (9.8, 15.5) | 2.2 (1.6, 3.1) | < 0.0001 |
| Year | Year-2018 | 209 | 3137 | 6.7 (5.8, 7.6) | 0. 6 (0.5, 0.7) | < 0.0001 |
| | Year-2019 | 138 | 1258 | 11.0 (9.3, 12.8) | 1.0 | |
| Scale | L | 81 | 1885 | 4.3 (3.4, 5.3) | 0.4 (0.3, 0.5) | < 0.0001 |
| | S | 266 | 2510 | 10.6 (9.4, 11.9) | 1 | |

L, farms had > 1000 lactation cows; S, farms had ≤ 1000 lactation cows

* $p < 0.05$ present significant difference

in fat content, lactose content and urea nitrogen between farms with a high prevalence and those with a low prevalence ($p > 0.05$) (Table 5).

## Discussion

In the current study, a good correlation was found between the results of a milk ELISA and a serum test for bTB in dairy cattle from Hubei province, China. This was in agreement with a previous study conducted in the Republic of Korea (Jeon et al. 2010). These results highlight that milk testing could complement testing of blood samples for bTB in surveillance programs. As the same ELISA test was used for sera and milk samples, it is not surprising that a high level of agreement was obtained. Undertaking a milk ELISA in conjunction with an intradermal test and interpreting the results in parallel would offer the advantage of an improved sensitivity. However, milk ELISA alone offers the advantage of easy collection and minimal stress to the sampled animal. According to previous studies, a serological response is improved if a skin test is administered more than 15 days prior to blood collection because of the anamnestic response. It is likely that a similar improvement in the sensitivity would be observed with milk ELISA test (Casal et al. 2014; Bridges and van Winden 2021). Further testing/evaluation of milk ELISA should be undertaken in known positive and negative animals to estimate and improve the test's accuracy, and to determine suitable cut-off points to maximise the test's diagnostic capability. Importantly, milk test offers the advantage of limiting stress on lactating cows (Parker et al. 2017) and also has the potential to be used on bulk milk samples to minimise testing costs (Villa-Mancera et al. 2018; Gates et al. 2021). However, as milk test is only suitable for lactating cows, it is obviously not suitable for testing males or non-lactating females.

**Table 4** bTB incidence rate using the milk antibody test for all 14 farms

| Farm | Incidence rate/ cow month | 95% CI[a] |
|---|---|---|
| A | 0.02 | 0.01, 0.03 |
| B | 0 | 0, 0.02 |
| C | 0.01 | 0, 0.01 |
| D | 0.03 | 0.01, 0.06 |
| E | 0.02 | 0.01, 0.03 |
| F | 0.03 | 0.02, 0.06 |
| G | 0.01 | 0, 0.04 |
| H | 0.01 | 0, 0.01 |
| I | 0.01 | 0, 0.03 |
| K | 0 | 0, 0.02 |
| L | 0 | 0, 0.02 |
| M | 0 | 0, 0.02 |
| N | 0.04 | 0.02, 0.07 |
| O | 0.03 | 0.01, 0.08 |
| Total | 0.01 | 0.008, 0.013 |

[a] 95% confidence intervals (CI) were calculated for each parameter using the method of Ross (Ross 2003)

There are very few reports on the prevalence and speed of transmission of bTB in China. In this study, we firstly reported the prevalence and incidence rate of bTB using a milk antibody ELISA, highlighting that the apparent (test) prevalence and incidence rate varied widely between sampled farms. These differences may be due to different management systems adopted, with some farms being closed herds not introducing any livestock. Some farms also regularly tested animals and removed test-positive cattle, whilst others did not adopt a routine

**Table 5** Milk quality and quantity on farms with different levels of bTB prevalence

| | Mean ± SD | | | | | | |
|---|---|---|---|---|---|---|---|
| **Prevalence level** | **Milk yield (kg/day)** | **Fat content (%)** | **Milk protein percentage (%)** | **Lactose content (%)** | **Total solids percentage (%)** | **SCC (× 1000 cell/mL)** | **Urea nitrogen content(%)** |
| Low | 25.4 ± 22.8 (24.4, 26.3) | 3.5 ± 1.9 (3.4, 3.6) | 3.3 ± 0.6 (3.3, 3.4) | 5.0 ± 0.5 (5.00, 5.04) | 12.4 ± 2.1 (12.4, 12.5) | 177.6 ± 1085.8 (132.3, 222.9) | 13.4 ± 4.6 (13.3, 13.6) |
| High | 21.9 ± 16.4 (21.3, 22.4) | 3.6 ± 2.4 (3.6, 3.7) | 3.5 ± 1.0 (3.45, 3.51) | 5.0 ± 0.7 (4.97, 5.02) | 12.6 ± 2.8 (12.5, 12.7) | 475.8 ± 2514.6 (392.3, 559.4) | 14.3 ± 27.2 (13.4, 15.2) |
| *p* value | < 0.01 | 0.07 | < 0.01 | 0.06 | 0.01 | < 0.01 | 0.14 |

*SCC* Somatic cell counts

testing regime. Our previous study, using blood samples, showed similar results with animal level prevalences varying from 0 (95% CI: 0.0, 0.0) to 59.5% (95% CI: 48.2, 70.0) and incidence rates from 0.03 head (95% CI: 0.01, 0.05) to 2.69 head (95% CI: 1.59, 3.50)/cow-month in different herds (Chen et al. 2018).

In addition, the current study reports bTB milk antibody levels had a negative correlation with milk yield and a positive correlation with SCC, MPP, lactose content and TS. These would result in a sizeable economic loss for the individual farms as well as the local dairy industry. A SCC threshold of 100,000 cells/mL was used to differentiate subclinical mastitis with inflammatory response of the mammary gland from non-mastitic healthy cows (Schwarz et al. 2010). The increased SCC in bTB positive animals might signify that bTB may be a cause of bovine mastitis. The role of bTB in bovine subclinical mastitis is further supported by their significant effects on milk production including milk yield and milk composition, including decrease in daily milk yield and lactose, and an increase in protein in high bTB prevalence farms, which also agreed with the findings of previous studies (Hernandez and Baca 1998; Barlowska et al. 2009). Unfortunately, in the current study, no further tests were undertaken and no information on environmental mycobacteria were available, and detailed data of on-farm management and husbandry factors was not collected. It is possible that the differences in milk quality parameters may have been affected by other on-farm factors that were not measured. Future studies should be conducted to expand the current study and collect more data on routine management and husbandry practices adopted on sampled farms.

To reduce the risk of human tuberculosis infection and to improve productivity, bTB should be controlled or eradicated. In 2012, China launched a control and eradication program for bTB in dairy cattle with an aim to eradicate the disease by 2020. However, our data indicates that only a few farms have met the criteria to be classified as bTB free, indicating that the disease has not been effectively controlled in dairy herds in the country. This lack of control is likely associated with the potential rapid transmission of the bacterium (Chen et al. 2018) and a failure to implement effective control measures. To control bTB, besides testing and culling positive animals in a timely manner, strict movement restrictions are critical to prevent movement of potentially infected animals to herds/districts with a low prevalence (Tweddle and Livingstone 1994; Max et al. 2011; Birch et al. 2018). There is also an urgent need for enhanced farm level biosecurity practices and strict control and supervision of the disposal of infected animals to prevent them from entering other herds. These measures, along with payment of market value compensation for infected animals, should be considered to achieve the ultimate goal of bTB freedom (Boukary et al. 2011; Enticott et al. 2015; Broughan et al. 2016; O'Hagan et al. 2016).

## Conclusion

This study identified a good agreement between milk and serum antibody test for bovine tuberculosis with a kappa value of 0.862 (95% CI: 0.7, 1.0; $p < 0.001$). There was a high prevalence and incidence rate for bTB in dairy herds according to the milk test in Hubei province. A strong association between bTB and reduced milk yield was found with elevated MPP, TS and SCC. This study highlights the advantages of using a more convenient and non-invasive test for the surveillance for bTB which has the potential to assist in the rapid diagnosis of the disease, and suggests to enhance the surveillance and screen herds using a combination of milk antibody testing with the intradermal test, and hence obtain more accurate estimate of the bTB situation and facilitate its control in China.

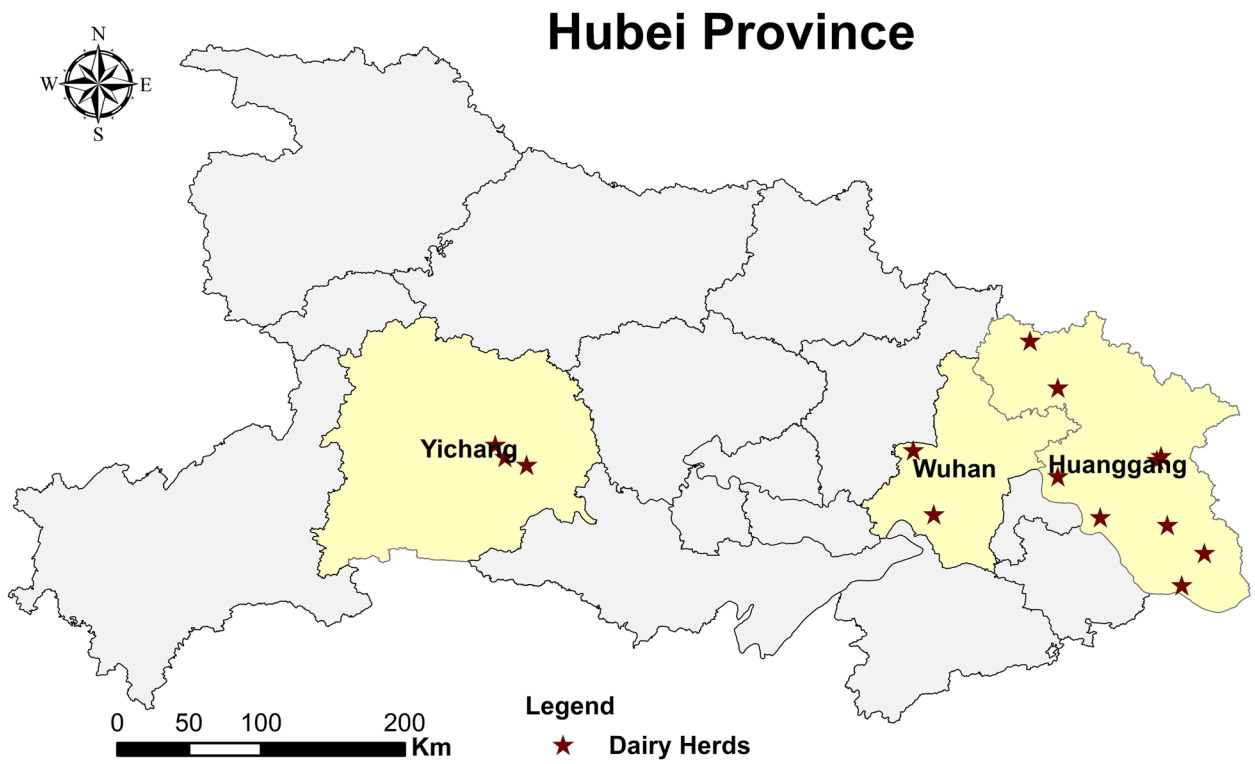


**Fig. 1** Location of 15 dairy farms of Hubei Province used in this study. Hubei province is located in the middle reaches of the Yangtze River in central China

## Methods

### Study area and samples

Both milk and blood samples were collected from 85 lactating cows in three dairy farms (B, D, I) in Hubei Province to determine the agreement of two tests. Farms were selected based on convenience (close to the State Key Laboratory of Agricultural Microbiology and cooperative owners) and size (≥ 100 lactating cows). At least 20 lactating cows free from mastitis and in the mid-stage of lactation (101–200 days after delivery) were selected from each farm for inclusion in the study.

The milk test was then used to determine the apparent (test) prevalence and incidence of bTB in 15 commercial dairy farms in Hubei province (Fig. 1) representing all dairy farms with > 100 lactating dairy cattle participating in the Hubei DHI program. This program involves the monthly collection and testing of individual cow milk samples to monitor milk composition and yield. Farms with ≤ 1000 cows in lactation were classified as small scale farms, and those with > 1000 cows as large scale. Samples were collected as previously reported (Wang et al. 2020).

A total of 1196 milk samples were collected from 11 dairy farms in January 2018, 1319 milk samples from 13 farms in April 2018, 622 milk samples from six farms in July 2018, 711 milk samples from nine farms in March 2019, and 547 milk samples from seven farms in June 2019. Sampling and herd classification information is presented in Table 6. No prior information was available on the prevalence of bTB on the sampled farms.

### Milk and serum tests for bovine tuberculosis

Milk and serum samples were treated as previously reported (Wang et al. 2020). Commercial bTB ELISA antibody detection kits using antigen MPB70/MPB83/CFP-10/ESAT were purchased from Wuhan Keqian Biology Co., Ltd with a manufacturer reported 72.37% (95% CI: 60.91, 82.01) sensitivity and 89.67% (95% CI: 86.61. 92.23) specificity (CI were calculated from the paper's data) (Wu et al. 2007). The serum antibody tests were conducted as per the manufacturer's instructions. Milk samples were tested following the same protocol as for sera except for a difference in sample dilution. The serum samples were diluted 50 times while the milk samples were not diluted. A farm was categorized as positive if one or more milk sample(s) from that farm tested positive.

Cows that were tested at least twice and were test negative at the very beginning in our current study were used for the incidence rate calculation. Totally, 2,794 cows

**Table 6** Description of sampling undertaken in the study

| Farm | Scale[a] | Tested number | | | | |
|---|---|---|---|---|---|---|
| | | 2018. 01 | 2018. 04 | 2018. 07 | 2019. 03 | 2019. 06 |
| A | L | 197 | 157 | 94 | 71 | 71 |
| B | L | 115 | 103 | 61 | 130 | - |
| C | L | - | 154 | 114 | 71 | 71 |
| D | L | - | 155 | 180 | - | 141 |
| E | S | 126 | 105 | 74 | 105 | 36 |
| F | S | 97 | 75 | - | - | - |
| G | S | 99 | 78 | - | - | - |
| H | S | 80 | 146 | - | - | - |
| I | S | 139 | 92 | - | - | - |
| J | S | 53 | 51 | - | - | - |
| K | S | 138 | - | 99 | 71 | 71 |
| L | S | - | 76 | - | 64 | - |
| M | S | - | - | - | 92 | 94 |
| N | S | 91 | 82 | - | 63 | 63 |
| O | S | 61 | 45 | - | 44 | - |
| Total | | 1196 | 1319 | 622 | 711 | 547 |

[a] L represents large scale farms (> 1000 lactating cows), S represents small scale farms (≤ 1000 lactating cows)

were tested , among that, 1,339 were tested at least twice and 97 were tested positive at the very beginning. Those cows were then removed from the subgroup used to calculate the incidence rate. Then 1,242 cows were then used for the calculation of incidence rate. Sample information is listed in Supplementary Table 1.

#### Milk quality and bTB antibody correlation

Data/information on individual cow milk yield and milk quality (percentages of fat, protein, lactose, total solids, and urea nitrogen in the milk, and SCC were measured using a CombiFoss FT + milk composition and somatic cell analyzer were provided by DHI. Farms were divided into two groups according to the lactoprevalence: high prevalence group (≥ 3%) and low prevalence group (< 3%) as a prevalence of 3% prevalence is the official threshold for implementing an on-farm TB control program in China (http://www.moa.gov.cn/nybgb/2017/dqq/201712/t20171230_6133930.htm). Complete information on milk quality and milk antibody test was available for 773 individual cows from 11 farms. Of these 773, 18 had been tested four times, 9 3 times, 581 twice and 165 once (total of 1426 milk samples tested).

#### Statistical analyses

Cohen's Kappa statistic and agreement were calculated to determine the relationship between milk and serum antibody test results using the software Epitools (https://epitools.ausvet.com.au/comparetwotests). Kappa values range from -1 to 1. When Kappa = 1, perfect agreement exists; When Kappa = 0, agreement is the same as would be expected by chance; When Kappa < 0, agreement is weaker than expected by chance. The higher the Kappa, the stronger the agreement and more reliable.

For calculating the incidence rate, only cows test-negative on the first test were considered at risk of becoming infected, and the incidence rate was calculated as new cases that divided by the animal time at risk (Dufour et al. 2012). 95% confidence intervals (CI) were calculated for each parameter using the method of Ross (Ross 2003). Test prevalence calculation followed previous studies (Thrusfield 2018, Wang et al. 2020).

The Chi-square test or Fisher's exact test were used to determine the relationship between farm scale and apparent prevalence. The Kolmogorov–Smirnov test was used to confirm data were normally distributed. The T-test was used to assess the significance of lactoprevalence on milk quality (Mean ± 2SD). Odds ratios and their 95% CI were calculated to evaluate the effect of sampling time on lactoprevalence (< 3% vs ≥ 3%).

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-023-00069-9.

**Additional file 1: Table S1.** Description of sampling for incidence ratecalculation.

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Not applicable

**Authors' contributions**
Conception and design of the study, YC, IR and AG; acquisition of data, SC, GW and XW; analysis and interpretation of data, YC, YW and IR; drafting and revising the article, YC and IR; final approval of the version to be submitted, AG. The authors read and approved the final manuscript.

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**Availability of data and materials**
Not applicable.

## Declarations

**Ethics approval and consent to participate**
The animal experiment plan was approved by the Huazhong Agricultural University Animal Experimental Ethics Committee under the protocol number HZAUCA-2019–006.

**Consent for publication**
Not applicable.

**Competing interests**
The author declares that he/she has no competing interests.

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**References**

Barlowska, J., Z. Litwiniczuk, A. Wolanciuk, and A. Brodziak. 2009. Relationship of somatic cell count to daily yield and technological usefulness of milk from different breeds of cows. *Polish Journal of Veterinary Science* 12 (1): 75–79.

Bauman, C.A., A. Jones-Bitton, J. Jansen, D. Kelton, and P. Menzies. 2019. Evaluation of bulk tank milk PCR and bulk tank milk modified ELISA tests for the detection of paratuberculosis at the herd level in goat and sheep dairies in Ontario. *Canada. Journal of Dairy Science* 102 (1): 511–520.

Birch, C.P.D., A. Goddard, and O. Tearne. 2018. A new bovine tuberculosis model for England and Wales (BoTMEW) to simulate epidemiology, surveillance and control. *BMC Veterinary Research* 14 (1): 273.

Boukary, A.R., E. Thys, E. Abatih, D. Gamatie, I. Ango, A. Yenikoye, and C. Saegerman. 2011. Bovine tuberculosis prevalence survey on cattle in the rural livestock system of Torodi (Niger). *PLoS ONE* 6 (9): e24629.

Bridges, N., and S. van Winden. 2021. The occurrence of *Mycobacterium avium* subspecies paratuberculosis positive milk antibody ELISA results in dairy cattle under varying time periods after skin testing for bovine tuberculosis. *Animals (basel)* 11 (5): 1224.

Broughan, J.M., D. Maye, P. Carmody, L.A. Brunton, A. Ashton, W. Wint, N. Alexander, R. Naylor, K. Ward, A.V. Goodchild, S. Hinchliffe, R.D. Eglin, P. Upton, R. Nicholson, and G. Enticott. 2016. Farm characteristics and farmer perceptions associated with bovine tuberculosis incidents in areas of emerging endemic spread. *Preventive Veterinary Medicine* 129: 88–98.

Casal, C., A. Diez-Guerrier, J. Alvarez, S. Rodriguez-Campos, A. Mateos, R. Linscott, E. Martel, J.C. Lawrence, C. Whelan, J. Clarke, A. O'Brien, L. Dominguez, and A. Aranaz. 2014. Strategic use of serology for the diagnosis of bovine tuberculosis after intradermal skin testing. *Veterinary Microbiology* 170 (3–4): 342–351.

Chen, Y., J. Wang, L. Yang, I. Robertson, H. Chen, and A. Guo. 2018. An intensive study of 4 dairy farms in China to determine the incidence rate of bTB. *Journal of Bacteriology and Mycology* 5 (8): 1087.

Cho, Y.S., S.C. Jung, and S. Oh. 2015. Diagnosis of bovine tuberculosis using a metal oxide-based electronic nose. *Letters in Applied Microbiology* 60 (6): 513–516.

Dufour, S., I.R. Dohoo, H.W. Barkema, L. Descoteaux, T.J. Devries, K.K. Reyher, J.P. Roy, and D.T. Scholl. 2012. Manageable risk factors associated with the lactational incidence, elimination, and prevalence of Staphylococcus aureus intramammary infections in dairy cows. *Journal of Dairy Science* 95 (3): 1283–1300.

Enticott, G., D. Maye, P. Carmody, R. Naylor, K. Ward, S. Hinchliffe, W. Wint, N. Alexander, R. Elgin, A. Ashton, P. Upton, R. Nicholson, T. Goodchild, L. Brunton, and J. Broughan. 2015. Farming on the edge: Farmer attitudes to bovine tuberculosis in newly endemic areas. *Veterinary Record* 177 (17): 439.

Evermann, J.F., D.M. DeAvila, S.M. Parish, C.H. Merritt, K.C. Noble, S. Srikanth, and A.L.E. Bronowski. 2019. Evaluation of a serum ELISA for detection of bovine leukemia viral antibodies in milk samples. *Journal of Veterinary Diagnostic Investigation* 31 (4): 598–600.

Gates, M.C., C.A. Evans, C. Heuer, H. Voges, and J.F. Weston. 2021. Temporal trends in bulk tank milk antibody ELISA and PCR test results for bovine viral diarrhoea in New Zealand pastoral dairy herds. *New Zealand Veterinary Journal* 69 (2): 73–82.

Hernandez, J., and D. Baca. 1998. Effect of tuberculosis on milk production in dairy cows. *Journal of the American Veterinary Medical Association* 213 (6): 851–854.

Jeon, B.Y., S.C. Kim, S. Je, J. Kwak, J.E. Cho, J.T. Woo, S. Seo, H.S. Shim, B.O. Park, S.S. Lee, and S.N. Cho. 2010. Evaluation of enzyme-linked immunosorbent assay using milk samples as a potential screening test of bovine tuberculosis of dairy cows in Korea. *Research in Veterinary Science* 88 (3): 390–393.

Katale, B.Z., E.V. Mbugi, S. Kendal, R.D. Fyumagwa, G.S. Kibiki, P. Godfrey-Faussett, J.D. Keyyu, P. Van Helden, and M.I. Matee. 2012. Bovine tuberculosis at the human-livestock-wildlife interface: Is it a public health problem in Tanzania? A review. *Onderstepoort Journal of Veterinary Research* 79 (2): 463.

Max, V., L. Paredes, A. Rivera, and C. Ternicier. 2011. National control and eradication program of bovine tuberculosis in Chile. *Veterinary Microbiology* 151 (1–2): 188–191.

O'Hagan, M.J., D.I. Matthews, C. Laird, and S.W. McDowell. 2016. Farmers' beliefs about bovine tuberculosis control in Northern Ireland. *The Veterinary Journal* 212: 22–26.

Olea-Popelka, F., A. Muwonge, A. Perera, A.S. Dean, E. Mumford, E. Erlacher-Vindel, S. Forcella, B.J. Silk, L. Ditiu, A. El Idrissi, M. Raviglione, O. Cosivi, P. LoBue, and P.I. Fujiwara. 2017. Zoonotic tuberculosis in human beings caused by Mycobacterium bovis-a call for action. *The Lancet Infectious Diseases* 17 (1): e21–e25.

Parker, A.M., J.K. House, M.S. Hazelton, K.L. Bosward, J.M. Morton, and P.A. Sheehy. 2017. Bulk tank milk antibody ELISA as a biosecurity tool for detecting dairy herds with past exposure to Mycoplasma bovis. *Journal of Dairy Science* 100 (10): 8296–8309.

Romha, G., G. Gebru, A. Asefa, and G. Mamo. 2018. Epidemiology of Mycobacterium bovis and Mycobacterium tuberculosis in animals: Transmission dynamics and control challenges of zoonotic TB in Ethiopia. *Preventive Veterinary Medicine* 158: 1–17.

Ross, T.D. 2003. Accurate confidence intervals for binomial proportion and Poisson rate estimation. *Computers in Biology and Medicine* 33 (6): 509–531.

Schwarz, D., U.S. Diesterbeck, K. Failing, S. Konig, K. Brugemann, M. Zschock, W. Wolter, and C.P. Czerny. 2010. Somatic cell counts and bacteriological status in quarter foremilk samples of cows in Hesse, Germany–a longitudinal study. *Journal of Dairy Science* 93 (12): 5716–5728.

Sibhat, B., K. Asmare, K. Demissie, G. Ayelet, G. Mamo, and G. Ameni. 2017. Bovine tuberculosis in Ethiopia: A systematic review and meta-analysis. *Preventive Veterinary Medicine* 147: 149–157.

Thrusfield, M. 2018. *Veterinay epidemiology*, 4th ed. UK: University of Edinburgh, Wiley Blackwell.

Tweddle, N.E., and P. Livingstone. 1994. Bovine tuberculosis control and eradication programs in Australia and New Zealand. *Veterinary Microbiology* 40 (1–2): 23–39.

Villa-Mancera, A., C. Pastelin-Rojas, J. Olivares-Perez, A. Cordova-Izquierdo, and A. Reynoso-Palomar. 2018. Bulk tank milk prevalence and production losses, spatial analysis, and predictive risk mapping of Ostertagia ostertagi infections in Mexican cattle herds. *Parasitology Research* 117 (5): 1613–1620.

Wang, Y., I.D. Robertson, S. Cheng, Y. Wang, L. Hou, G. Wang, X. Wu, X. Li, Y. Chen, and A. Guo. 2020. Evaluation of a milk ELISA as an alternative to a serum ELISA in the determination of the prevalence and incidence of brucellosis in dairy herds in Hubei Province. *China. Preventive Veterinary Medicine* 182: 105086.

Wu, B., S. Zhang, Q. Deng, J. Xiang, J. Chen, Z. Liu, X. Chen, Y. Han, B. Zhao, H. Chen, and A. Guo. 2007. Application of the specifici tetra-valent fusion protein of Mycobacterium bovis in the diagnosis for bovine tuberculosis. *Chinese Journal of Zoonoses (In Chinese)* 11: 1123–1126.