Research Article

An Intensive Study of 4 Dairy Farms in China to Determine the Incidence Rate of bTB

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Abstract

Bovine Tuberculosis (bTB) is endemic in most parts of China, with epidemics occurring in some parts of the country. However little is currently known about the epidemiology of the disease. This research describes the occurrence of bTB in four dairy farms in China. The true animal level prevalence varied from 0.0% (95% CI: 0.0, 0.0) to 59.5% (95% CI: 48.2, 70.0). The incidence rate highlighted the rapid transmission between animals in herds ranging from 0.03 (95% CI: 0.01, 0.05)/cow-month in a closed farm which culled IFN-γ release assay test-positive animals to 2.69 (95% CI: 1.59, 3.50)/cow-month in a closed farm which culled animals positive to both the PPD skin test and IFN-γ release assay.

Keywords: Incidence Rate; Bovine Tuberculosis; Transmission; China

Introduction

Bovine Tuberculosis (bTB) is a widely distributed chronic bacterial disease caused by any of the disease-causing mycobacterial species especially *Mycobacterium bovis* (*M. bovis*) within the *Mycobacterium tuberculosis*-complex. Cattle is the natural host of *M. bovis*, which can also infect other domestic and wild animals as well as humans [1], and can be transmitted among or within different species such as cattle and humans by direct or indirect contact [2].

In 20th Centry 1990s, cattle were State-owned assets in China, at that period of time, bovine Tuberculosis (bTB) was controlled and kept in a very low level. As the privatization of cattle, bTB was epidemic in most area in China. Not until 2012, Chinese government began with the animal infectious diseases eradication program, regarded bTB as the priority animal diseases in China. According to the program, all cattle herds should be routinely tested, positive cattle (reactors) should be slaughtered. Before the eradication program, epidemiological characteristic and dynamics of bTB, including incidence rate and prevalence of bTB would be essential for the controlling strategies.

In many epidemiological research, dynamic modelling was widely used to estimate the bTB infection especially within-herd transmission rates because of the long incubation periods of bTB [3-5]. But the results varied a lot according to different mathematical models, management practices, species types or other factors.

As different areas had different breeding modes, in the current work, we selected 4 farms of two typical kinds of modes in developed and less developed area in China, which represented closed and open farms, respectively. Traced the farms for 2-7 years, firstly evaluated the bTB prevalence, then used those parameters to estimate the incidence rate of infection, in order to estimate the bTB transmission in Chinese herds. The IFN-γ release assay, recommended by the OIE, was used to test for bTB in this study [6].

Materials and Methods

Farms and animals

Four farms were included in this study. Farm A, located in Hubei Province in central China, which regarded as less developed area, purchasing cows from other dairy farms. Test positive cows were removed from the herd within two weeks of obtaining a positive result and were replaced with Single Intradermal Comparative Cervical Test (SICCT, PPD skin test) -negative animals sourced from other commercial dairies. Because of the frequently purchased and selling of the farm, data for Farm A were available for 2010 to 2016, with all cows tested in Feb. 2010, Sep. 2011, Dec. 2012, Jan. 2013, Dec. 2014, July. 2015 and Mar. 2016. The number of animals tested at each point varied, with a starting number of 141 cows on Feb. 2010, then all cows were removed from that farm and 195 cows were newly introduced and tested on Sep. 2011; 49 new cows were introduced after that and 84 in total were tested on Dec. 2012; On Jan. 2013, we found 93 new cows and tested 126 cows in total; then 129 cows were introduced and 236 were tested in total on Dec. 2014; Only 5 were introduced after that and 57 were tested on July. 2015, on Mar. 2016, 212 cows were tested with 16 new comers.

Farms B and C, located in Shanghai City in East China, which regarded as developed area, are only physically separated by a narrow 1-meter wide pathway. They were originally owned by the same company, although were subsequently sold to different farmers. Both farms are closed farms neither purchasing cows nor selling cows from or to other dairies, so the two farms are not really separate epidemiological units. All cows were tested by SICCT on Oct. 2016,

Table 1: Apparent Prevalence (AP) of bovine tuberculosis on 4 dairy farms in China.

Year/Month	2010.2		2011.9		2012.12		2013.1		2014.12		2015.7		2016.3	
Farm	AP	95% CI	AP	95% CI	AP	95% CI	AP	95% CI	AP	95% CI	AP	95% CI	AP	95% CI
A	27% (38/141)	19.8,35.1	8.2% (16/195)	4.8, 10.3	54.8% (46/84)	43.5, 65.7	27.8% (35/126)	20.2, 36.5	19.5% (46/236)	14.6, 25.1	49.1% (28/57)	35.6, 62.7	19.8% (42/212)	14.7, 25.8
A (new cows)	N/A	N/A	8.1% (16/195)	4.812.98	36.7% (18/49)	23.4, 51.7	30.1% (28/93)	21.0, 40.5	13.2% (17/129)	7.9, 20.3	0.0% (0/5)	0.0, 52.2	0.0% (0/16)	0.0, 20.6
Year/Month	2016.1		2016.12		2017.2		2017.9							
Farm	AP	95% CI	AP	95% CI	AP	95% CI	AP	95% CI						
B	1.3% (6/477)	0.5, 2.7	1.5% (7/477)	0.6, 3.0	38.8% (125/322)	33.5, 44.4	35.0% (72/206)	28.5, 41.9						
C	2.3 (8/355)	1.0, 4.4	0.8 (3/355)	0.2, 2.4	32.1 (54/168)	25.2, 39.8	24.3 (34/140)	17.4, 32.2						
Year/Month	2011.6		2012.7											
Farm	AP	95% CI	AP	95% CI										
D	14.5% (69/477)	11.4, 17.9	5.8% (23/394)	3.7, 8.6										
D (new cows)	N/A	N/A	12.1% (15/124)	6.9, 19.2										

Table 2: Estimated Real Prevalence (RP) of bovine tuberculosis on four dairy farms in China.

Year/Month	2010.2		2011.9		2012.12		2013.1		2014.12		2015.7		2016.3	
Farm	RP	95% CI	RP	95% CI	RP	95% CI	RP	95% CI	RP	95% CI	RP	95% CI	RP	95% CI
A	26.10%	19.1, 34.1	3.60%	1.4, 0.1	59.50%	48.2, 70.0	27.10%	19.5, 35.7	17.10%	12.5, 22.5	52.70%	39.0, 66.1	17.50%	12.6, 23.3
A (new cows)	N/A	N/A	3.60%	1.4, 0.1	37.80%	24.4, 52.8	29.90%	20.8, 40.2	9.50%	5.1, 16.0	0.00%	0.0, 0.0	0.00%	0.0, 0.0
Year/Month	2016.1		2016.12		2017.2		2017.9							
Farm	RP	95% CI	RP	95% CI	RP	95% CI	RP	95% CI						
B	0.00%	0.0, 0.0	0.00%	0.0, 0.0	40.30%	34.9, 45.9	35.70%	29.1, 42.6						
C	0.00%	0.0. 0.0	0.00%	0.0, 0.0	32.30%	25.3, 39.9	22.90%	16.2. 30.7						
Year/Month	2011.6		2012.7											
Farm	RP	95% CI	RP	95% CI										
D	11.10%	8.4, 14.3	0.80%	0.1, 2.2										
D (new cows	N/A	N/A	8.20%	4.1, 14.6										

Dec. 2016, Feb. 2017 and Sep. 2017 with Farm B initially having 477 cows on Oct. and Dec. 2016, 322 on Feb. 2017 and 206 on Sep. 2017. For Farm C 355 were tested on Oct. and Dec. 2016, 168 on Feb. 2017, 140 on Sep. 2017. No cows were introduced to these two farms during our observation. Before our 1st test (Oct. 2016), ACDC in Shanghai city tested all cows on those two farms by tuberculin skin test and killed all positive-reaction cows.

Farm D, located in Jiangsu Province which was also developed area in East China, also purchased cows directly from other dairy farms. In this study cows were only tested twice, on June 2011 when 477 cows were tested, and on July 2017 when 394 were tested with 124 new cows. They killed all positive animals tested by BOVIGAM.

In those closed farms, positive cows were slaughtered after tested, so cannot be traced for a long time.

Bovine tuberculosis detection

Cows were tested using the commercial BOVIGAM *Mycobacterium bovis* Gamma Interferon Test Kit for Cattle (Prionics, Switzerland) according to the manufacturer's instructions. Briefly blood samples were divided into three wells (each well containing 1.5 ml of blood). Then all three wells were mixed with 100μl PPD-B, PPD-A and PBS respectively, and then cultured overnight at 37°C in an incubator containing 5% CO_2. The supernatants from each well were then harvested and the presence of IFN-γ confirmed with a sandwich ELISA. A result was considered as positive if $OD_{PPD\text{-}B} - OD_{PPD\text{-}A} \geq 0.1$ and $OD_{PPD\text{-}B} - OD_{PBS} \geq 0.1$, and negative if $OD_{PPD\text{-}B} - OD_{PPD\text{-}A} < 0.1$ and $OD_{PPD\text{-}B} - OD_{PBS} \geq 0.1$.

Data analysis

To evaluate the frequency of bTB, the test and real prevalence and incidence rate were calculated. For incidence, only cows that were negative and then became positive on a subsequent test were included in the analyses. 95% confidence intervals were calculated for each parameter.

Apparent (test) Prevalence (AP) = number of cows test positive ÷ total number tested

Real prevalence = (AP + Specificity -1) ÷ (Sensitivity + Specificity-1)

Incidence rate = new cases ÷ animal time at risk.

Results

Cattle bTB prevalence on individual farms

The test results (AP) of the IFN-γ test for bTB in each farm are presented in (Table 1). The AP varied from 8.2% (95% CI: 4.8, 10.3) to 54.8% (95% CI: 43.5, 65.7) on Farm A during the 6 years of testing. As Farms A purchased cows from others, we also calculated the AP

Table 3: Incidence Rate (IR) for bovine tuberculosis on four dairy farms in China.

	2012.12		2013.1		2014.12		2015.7		2016.3		Total	
cow/year	**IR**	**95% CI**	**IR**	**95% CI**	**IR**	**95% CI**	**IR**	**95% CI**	**IR**	**95% CI**	**IR**	**95% CI**
Farm A	0.43	0.30, 0.57	0.14	0.07, 0.22	0.03	0.01, 0.05	0.04	0.01, 0.10	0.04	0.02, 0.08	0.29	0.23, 0.35
	2016.12		**2017.2**		**2017.9**						**Total**	
	IR	**95% CI**	IR	**95% CI**	IR	**95% CI**					**IR**	**95% CI**
Farm B	0.09	0.04, 0.18	2.69	1.59, 3.50	0.4	0.32, 0.49					0.86	0.80, 0.90
Farm C	0.05	0.01, 0.14	1.86	1.48, 2.52	0.32	0.26, 0.42					0.55	0.47, 0.63
	2012.7											
	IR	**95% CI**										
Farm D	0.03	0.01, 0.05										

of cows that newly introduced to that farms in separated time points. Results showed that only in introduced cows, the highest AP was up to 36.7% (95% CI: 23.4, 51.7) on Dec. 2012 and 30.1% (95% CI: 21.0, 40.5) on Jan. 2013.

AP in Farms B and C followed similar patterns with low AP's of 1.3% (95% CI: 0.5, 2.7) and 0.8% (95% CI: 0.2, 2.4), respectively and high AP of 38.8% (95% CI: 33.5, 44.4) and 32.1% (95% CI: 25.2, 39.8), respectively. Cows on Farm D, which were only sampled twice, had an AP decrease from 14.5% (95% CI: 11.4, 17.9) to 5.8% (95% CI: 3.7, 8.6) over the 13-month sampling period; for the introduced ones, AP was 12.1% (6.9, 19.2) on July. 2012.

The real prevalence for each sampling was calculated using a sensitivity and specificity of the IFN-γ test of 88% and 95% [7] (Table 2). RP varied from 3.6% (95% CI: 1.4, 0.1) to 59.5% (95% CI: 48.2, 70.0) on Farm A during the 6 years of testing, in the new introduced cows, RP was up to 37.8% (95% CI: 24.4, 52.8) on Dec. 2012 and to 29.9% (95% CI: 20.8, 40.2) on Jan. 2013.

RP in Farms B and C were regarded as TB free at the first two tests in 2016 (upper 95% CI was 0%), but increased dramatically to 40.3% (95% CI: 34.9, 45.9) and 32.3% (95% CI: 25.3, 39.9), respectively on Feb. 2017 before reducing slightly to 35.7% (95% CI: 29.1, 42.6) and 22.9% (95% CI: 16.2, 30.7), respectively seven months later. RP on Farm D decreased from 11.1% (95% CI: 8.4, 14.3) to 0.8% (95% CI: 0.1, 2.2) over the 13-month period, and RP for new introduced cows was 8.2% (95% CI: 4.1, 14.6) on July. 2012.

Incidence rate on individual farms

The incidence rates for the four farms are summarized in (Table 3). Over the four year period the total incidence rate was 0.29/cow-year (95% CI: 0.23, 0.35) on Farm A. On individual tests the incidence rate varied from 0.03 (95% CI: 0.01, 0.06) to 0.43/cow-year (95% CI: 0.30, 0.57).

On Farm B the incidence rate over the study period was 0.86/cow-year (95% CI: 0.81, 0.90), ranging from 0.09 (95% CI: 0.04, 0.18) to 2.69/cow-year (95% CI: 1.59, 3.50). For Farm C, the overall incidence rate was 0.55/cow-year (95% CI: 0.47, 0.63), ranging from 0.05 (95% CI: 0.01, 0.15) in Dec. 2016 to 1.86/cow-year (95% CI: 1.48, 2.52) in Feb. 2017.

On Farm D only two tests were conducted, with a calculated incidence rate of 0.03/cow-year (95% CI: 0.012, 0.054).

Discussion

In China, even though the bTB controlling program has been carried out at national level for almost 6 years, the achievement is far away from the original plan. Given the situation, knowledge of the occurrence especially the dynamics of bTB spread within Chinese herds is essential.

In the current study, the presence of bTB could have serious implicates on dairy cows even human health. Although very rare data was published for cow tuberculosis in China to our knowledge, as our results reported the highest real prevalence of bTB of 59.5% (95% CI: 48.2, 70.0) in a dairy from Hubei Province, and the lowest (0.0%; 95% CI: 0.0, 0.0) in Shanghai City which was considered free from disease at that sampling, we can confirm that bTB was seriously epidemic in part of China, and the epidemiological status varied a lot in different regions.

According to the mathematical models for bTB transmission, residual infection and introduction of infected cattle from other herds, contiguous spread from infected neighbor herds are very important causes for that disease [8]. In this study, Farm A and D introduced cows from others, based on the related disciplines in China, all newly introduced cows should be tested negative before inducing, but according to our test, the new cows suffered a very high real prevalence even up to 37.8% (95% CI: 24.4, 52.8) in Farm A on Dec. 2012 and 8.2% (95% CI: 4.1, 14.6) on Jul. 2012 in Farm D; there were two possibilities, first, those new cows were not completely detected or the test method was not accurate enough, which means, the introduced cows were infected cows before inducing; second, all new cows were TB free before inducing, but were infected after inducing.

In China, single intradermal comparative cervical tuberculin skin test was commonly used for bTB test, which had a sensitivity of 50% (95% CI: 0.26, 0.78) [9], and lead to an inaccurate result; the presence of latent infections makes the control of bTB challenging, and even the introduction of test-negative animals will not guarantee freedom of disease introduction due to the potential for infection to be incubating in test-negative animals. As it was reported that introduction of infected animals has been reported to be responsible for 84% of newly infected farms by a dynamic model [10], to repeat negative testing was urgently needed.

By the other side, quickly transmission speed was also an important

factor for TB infection. In the current study, Bovigam, which has a high reported sensitivity of between 81.8 and 100% and specificity between 94 and 100% [11] was used to determine the incidence rate of bTB. By using that test, we found a high speed of transmission in farms. For Farm A, an overall high incidence rate of 0.29/cow-year was obtained. Considered the tuberculin skin test was not accurate enough, there is potential for many false test-negative cows to remain in the herd, resulting in subsequent spread of infection.

Even though Farms B and C were closed farms, and all PPD skin test positive cows were killed before our first test, transmission of infection was still fast, 0.86/cow year (95% CI: 0.80, 0.90) in total in Farm B, and 0.55/cow year (95% CI: 0.47, 0.63) in total in Farm C. To analyzed the possible factor, we traced the test positive cows for their mothers, and we found that many of the animals test-positive in Feb. 2017 were only 1 year old, whose mothers had previously been culled because of a positive test. As bTB can be transmitted through raw milk [12], we may supposed that it is important to identify calves of infected dames and remove them at the same time as their mothers.

For Farm D, transmission of infection was obtained as 0.03/cow-year (95% CI: 0.01, 0.05). Although Farm D introduced cows, too, they used Bovigam which was accurate enough to test all cows and killed all positive ones, so it resulting in a significantly lower prevalence, and a rate of disease spread (incidence).

What's more, government compensation also a very important factor for bTB controlling. Here we selected both developed and developing area, they have different financial supports of local government, for developed area, local government provides 80% market price for bTB positive cow's culling, with commercial farm insurance of cows, so the farmer are willing to weed out diseased cows; but for the developing ones, most of them don't have commercial farm insurance, and the local government paid very little for culling positive animal, in that case, most farmers are reluctant to culling, which leads to conceal or contradict for testing.

Conclusion

bTB was demonstrated to be quite seriously epidemic in some farms in China, and this disease can spread quickly in both open and closed dairy farms. Inducing infected animals was a very dangerous factor for bTB transmission. Slaughter of test-positive animals is an effective way to control the disease when highly sensitive tests are used [13]. The gamma interferon test is an accurate test however it is expensive. In China, bTB is epidemic in most parts of the country and it is recommended that a test and slaughter campaign is implemented to reduce the level of disease rapidly to reduce productivity losses in the dairy industry and to reduce the danger of human infection from infected milk.

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Citation: Chen Y, Wang J, Yang L, Robertson ID, Chen H and Guo A. An Intensive Study of 4 Dairy Farms in China to Determine the Incidence Rate of bTB. J Bacteriol Mycol. 2018; 5(8): 1087.