Cross-Sectional Study on Swine Tuberculosis Prevalence in Gansu Province, China from February to June 2021

Liang Sun[1], Yao Xi[1], Jiaxin Huang[1], Jifei Yang[2], zengjun Lu[2], Pu Sun[1], and Qiaoying Zeng[1]

[1]Gansu Agricultural University
[2]Lanzhou Veterinary Research Institute

April 16, 2024

Abstract

Summary: Tuberculosis (TB), caused by the *Mycobacterium tuberculosis* complex (MTBC), poses a major global threat to the health of humans and animals. The Suidae family is highly susceptible to TB and is used as screening sentinels for testing the presence of MTBC in the environment, but swine TB (STB) tends to be neglected worldwide. China has the largest pig population in the world, but epidemiological information on STB is scarce. In this study, 1379 serum samples were randomly collected from 45 herds of Gansu province, China from February to June 2021 and tested with PPD-B-ELISA. The STB prevalence at individual level was 0.22% (95% CI: 0.04%, 0.63%) and varied from 0.00% to 2.20% in different cities. The prevalence at herd level was 4.44% (95% CI: 0.54%, 15.15%) and varied from 0.00% to 33.33% in different cities. The STB prevalence in intensively raised three-crossbred pigs (0.23%, 95% CI: 0.05%, 0.68%) was higher than that in free-range raised Tibetan pigs (0.00%, 95% CI: 0.00%, 3.85%) ($p = 0.81$). Besides, 180 serum samples were collected from Guangxi province, China, and STB prevalence in Guangxi province (1.67%, 95% CI: 0.35%, 4.79%) was significantly higher than that in Gansu province ($p < 0.05$). These latest STB prevalence data are strongly suggested to conduct a randomized nationwide cross-sectional study on a regular basis for the development of an effective national program for STB surveillance and control.

Cross-Sectional Study on Swine Tuberculosis Prevalence in Gansu Province, China from February to June 2021

Liang Sun[1], Yao Xi[1], Jiaxin Huang[1], Jifei Yang[2], Zengjun Lu[2], Pu Sun[1, 2*], Qiaoying Zeng[1]11[*] Co-corresponding authors: Pu sun, Email: sunpu@caas.cn; Qiaoying Zeng, Email: zengqy@gsau.edu.cn.

[1] Laboratory of Veterinary Microbiology, College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, 730070, China.

[2] State Key Laboratory of Veterinary Etiological Biology, National Foot and Mouth Disease Reference Laboratory, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, 730070, China.

Running title: Cross-Sectional Survey on Seroprevalence of Swine Tuberculosis in Gansu Province, China

Corresponding authors: Qiaoying Zeng, Laboratory of Veterinary Microbiology, College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, 730070, China, Tel: +8613893316398, Email: zengqy@gsau.edu.cn (Q.Z); Pu Sun, State Key Laboratory of Veterinary Etiological Biology, National Foot and Mouth Disease Reference Laboratory, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, 730070, China, Tel: +8609318378501; Email: *sunpu@caas.cn* (P.S.).

Summary: Tuberculosis (TB), caused by the *Mycobacterium tuberculosis* complex (MTBC), poses a major global threat to the health of humans and animals. The Suidae family is highly susceptible to TB and is used as screening sentinels for testing the presence of MTBC in the environment, but swine TB (STB) tends to be neglected worldwide. China has the largest pig population in the world, but epidemiological information on STB is scarce. In this study, 1379 serum samples were randomly collected from 45 herds of Gansu province, China from February to June 2021 and tested with PPD-B-ELISA. The STB prevalence at individual level was 0.22% (95% CI: 0.04%, 0.63%) and varied from 0.00% to 2.20% in different cities. The prevalence at herd level was 4.44% (95% CI: 0.54%, 15.15%) and varied from 0.00% to 33.33% in different cities. The STB prevalence in intensively raised three-crossbred pigs (0.23%, 95% CI: 0.05%, 0.68%) was higher than that in free-range raised Tibetan pigs (0.00%, 95% CI: 0.00%, 3.85%) ($p = 0.81$). Besides, 180 serum samples were collected from Guangxi province, China, and STB prevalence in Guangxi province (1.67%, 95% CI: 0.35%, 4.79%) was significantly higher than that in Gansu province ($p < 0.05$). These latest STB prevalence data are strongly suggested to conduct a randomized nationwide cross-sectional study on a regular basis for the development of an effective national program for STB surveillance and control.

Keywords : Swine tuberculosis, Gansu province, China, Cross-sectional survey, Seroprevalence, Serology

Introduction

Tuberculosis (TB) is known as an important zoonotic infectious disease since humans and animals can be cross-infected by the*Mycobacterium tuberculosis* complex (MTBC). According to the Global Tuberculosis Report in 2021 by the World Health Organization, there were about 5.8 million new Human TB (hTB) cases and 1.5 million new deaths in 2020 (Global Tuberculosis Report in 2021). Although hTB is mainly caused by *Mycobacterium tuberculosis* (*M. tb*), 17.0%, 26.1%, 5.3%, and 6.5% of hTB cases have been reported to be caused by *Mycobacterium bovis* (*M. bovis*) in Ethiopia, Tanzania, Turkey, and the west bank of Palestine, respectively (Bayraktar et al., 2011; Ereqat et al., 2012; Fetene et al., 2011; Kazwala et al., 2001; Mfinanga et al., 2004; Shitaye et al., 2007).*M. bovis* has an extraordinary host range, including humans, cattle, pigs, and a variety of domesticated and wild animals (Cousins et al., 2005). In May 2014, the World Health Assembly passed a resolution of the post-2015 End TB Strategy with its ambitious targets to be fully realized in 2035 (Sun et al., 2021). However, MTBC infection in the multi-host system is a great barrier to achieving this goal worldwide.

The Suidae family has been reported to be highly susceptible to TB, and the detection of severe typical tubercles in pigs and wild boars suggests that those species may act as maintenance hosts rather than dead-end hosts (Di Marco et al., 2012; Naranjo et al., 2008). Due to the high susceptibility to MTBC, domestic pigs and feral swine are used as screening sentinels for testing the presence of MTBC in the environment (Bailey et al., 2013; L. A. Corner et al., 2015). Swine TB (STB) is a worldwide disease that is often neglected (Tbran et al., 2019). Therefore, as a reservoir for MTBC especially for *M. bovis* , swine might transmit MTBC to humans, especially in TB-endemic areas (Bollo et al., 2000; Nugent et al., 2015). STB has also been reported in other Suidae families such as bush pigs (*Potamochoerus porcus*) in South Africa (Hlokwe et al., 2014), warthogs (*Phacochoerus africanus*) in Africa (Miller et al., 2016), and collared peccaries (*Tayassu tajacu*) in South America (Mayer et al., 2012).

Proper identification of STB, followed by implementation of suitable management strategies, is important for control of STB (Csivincsik et al., 2016). However, there are limited STB diagnosis tests available. Mycobacterial culture remains the gold standard method for infection confirmation, but it is quite difficult due to several factors including the chronic process of TB, a high proportion of persistent infection, the slow growth of MTBC, low efficiency of isolation of MTBC, and the biosafety requirement for MTBC culture, etc (Boadella et al., 2011; L. A. L. Corner et al., 2012). Standard in vivo diagnosis methods including tuberculin skin tests and interferon-gamma release assays are mainly developed and calibrated in bovine TB, but these methods also have some limitations including multiple handling events required or time constraints since blood samples must be handled in the laboratory within 24 hours after collection (Buddle et al., 2009; Schiller et al., 2010). Alternatively, antibody-based diagnosis has been widely used for STB diagnosis owing to its convenience and good performance (Boadella et al., 2011; Gil et al., 2010; Pedersen et al., 2017; Perez de Val et al., 2017). Unlike many other animals, swine appear to produce detectable humoral responses soon after *M. bovis* infection, and the responses are maintained during the development of the disease (Garrido et al., 2011; Roos et al., 2016). This allows rapid detection of STB by serological assays (Boadella et al., 2011).

China has the largest pig population in the world, and the pig production industry is an important driver of the China economy. Sufficient data on STB prevalence are needed for the development of an effective STB surveillance and control program, but epidemiological information on STB in China is very scarce. Therefore, a cross-sectional study was conducted for assessing the STB prevalence in Gansu province in China with PPD-B-ELISA (de Val Perez et al., 2011; Garcia-Bocanegra et al., 2012; Gil et al., 2010).

Materials and Methods

Study area

Gansu province (located in Northwestern China) lies between 32°11'-42deg57' north latitude and 92deg13'-108deg46' east longitude with a total area of 425,800 square kilometers (Figure 1). Gansu has 12 prefecture cities and 2 autonomous prefectures.

Ethics Statement

The protocols regarding animal sampling were performed following the management guidelines of the Gansu Ethical Review Committee (license no. SYXK-89 GAN-2014-003). The study was explained to all the farm owners and oral consent of farms was obtained before sampling.

Animals and sampling

According to the data published by the Gansu Province Bureau of Statistics, there were 6.89 million swine in 2020 (Table 1). To obtain a sample size representative of the Gansu swine population, the number of pigs to be tested was calculated with EPITOOLS (*https://epitools.ausvet.com.au/oneproportion*). The minimum sample size was calculated to be 664 with an assumed STB prevalence of 50%, a confidence level of 99%, and desired precision of 5%. According to the ratio of the number of swine in each city to the number of swine in the province, the sample size was calculated and presented in Table 1.

Then, a cross-sectional multistage sampling strategy was used for selecting the study population. Firstly, scaled pig farm (intensively raised three-crossbred pig number [?] 200) was the primary sampling unit, and at least three farms were randomly selected in each city. It is worth noting that the pigs raised in Gannan Tibetan autonomous prefectures are free-range Tibetan pigs. Secondly, swine were the secondary sampling units, and at least 30 swine were randomly selected from each selected farm. Pigs were selected using systematic sampling, in which the first pig was randomly selected, and subsequent pigs were selected with a fixed sampling interval. In addition, there were 180 serum samples collected from Guangxi province.

Serological analysis

All the serum samples were tested in duplicate with PPD-B-ELISA as previously described (de Val Perez et al., 2011; Garcia-Bocanegra et al., 2012; Perez de Val et al., 2017). The 96-well plate was coated with 100 µL bovine PPD (PPD-B, Harbin Pharmaceutical Group Bio-vaccine Co. Ltd., China) which was prediluted with carbonate/bicarbonate buffer to a final concentration of 2 µg/mL and incubated overnight at 4°C. After 45-min blockade at 37°C with PBS containing 0.05% Tween 20 (PBST) and 0.5% casein, serum samples (diluted at 1/200 in PBST containing 1% casein) were added into 96-well plate in duplicate and incubated for 1 h at 37°C. Then, 96-well plate was added with protein A and protein G conjugated peroxidase (Sigma, America) and incubated for 1 h at 37°C. The protein A and protein G conjugated peroxidases were prediluted in PBST containing 1% casein to final concentrations of 50 ng/mL and 100 ng/mL, respectively. The plate was washed six times with PBST after each step during this procedure. Afterward, the plate was added with 100 µL TMB substrate (Beyotime, China) and incubated for 20 min in the dark, and then the reaction was stopped with 100 µL 1 N HCl. After incubation, the Optical Density at 450 nm (OD_{450}) was calculated as the sample OD_{450} value minus the background OD_{450} value (nonspecific absorbance in wells with no antigen added). A sample was defined as positive when the average OD_{450} value was higher than the cut-off value (calculated as the mean of the background OD_{450} plus 3 folds of standard deviation). All the positive sera and 20 randomly selected negative sera were further tested with PPD-A-ELISA which was the same as

PPD-B-ELISA but coated with avian PPD (PPD-A, Harbin Pharmaceutical Group Bio-vaccine Co. Ltd., China).

Statistical analyses

The farm was defined as positive if at least one swine was detected as positive. Individual prevalence was calculated as the ratio of the number of positive swine to the total number of swine. Similarly, herd prevalence was calculated as the number of positive farms to the total number of farms examined. The prevalence and the 95% confidence intervals were determined using the binomial distribution with Microsoft Excel 2016 (Microsoft Corporation, USA). OR and Fisher's exact test in SPSS 25 (International Business Machines Corporation, America) were used to determine the difference in positive and negative results between groups. $p < 0.05$ was considered as significantly different, and $p < 0.01$ as extremely significantly different.

Results

In total, 1379 serum samples from 45 herds were collected from February to June 2021 in Gansu province. On average, 98.5 serum samples (ranging from 90 to 150) were selected randomly from each city (Table 1). The overall STB prevalence at the individual level was 0.22% (95% CI: 0.04%, 0.63%) and varied from 0.00% to 2.20% in different cities (Table 1) (Figure 2). The prevalence at the individual level was 2.20% (95% CI: 0.27%, 7.71%) in Zhangye city, and that in Wuwei city was 1.11% (95% CI: 0.03%, 6.04%).

The overall STB prevalence at the herd level was 4.44% (95% CI: 0.54%, 15.15%) and varied from 0.00% to 33.33% in different cities (Table 1). Both Wuwei city and Zhangye city had one positive herd with a prevalence of 33.33% (95% CI: 0.84%, 90.57%) at the herd level.

The STB prevalence in intensively raised three-crossbred pigs (0.23%, 95% CI: 0.05%, 0.68%) was higher than that in free-range raised Tibetan pigs (0.00%, 95% CI: 0.00%, 3.85%), but the difference between them was not significant ($p = 0.81$). In addition, the STB prevalence in Guangxi province (1.67%, 3/180, 95% CI: 0.35%, 4.79%) was higher than that in Gansu province, and the difference between them was significant ($p < 0.05$) with odds ratio (OR) of 7.77 (95% CI: 1.56, 38.81).

Discussion

Although STB has not been subjected to the official investigation, there is a strong body of evidence that TB can be actively present in the pig population. Either in Spanish wild boars (Naranjo et al. 2008) or Nebrodi Black Pigs in Sicily (Di Marco et al., 2012), the high rates of reported granulomatous lesions related to TB suggest that suids can act as maintenance hosts. Furthermore, a novel strain of *M. bovis* was isolated from Nebrodi Black Pigs, arousing the concern of transmission of *M. bovis* genotypes in the porcine species (Amato et al., 2018; Di Marco et al., 2012). This implies that the control of STB should be mandatory if the presence of pigs poses an epidemiological risk.

The carcass inspection at the slaughterhouse, which is regulated by legislation, is not the best approach for STB prevention and control. It has been reported that the isolation of *M. bovis* does not necessarily correspond to visually detectable lesions at the abattoir (Garcia-Jimenez et al., 2013; Martin-Hernando et al., 2007). Besides, the organs from which *M. bovis* is most frequently isolated in swine are mandibular lymph nodes and tonsils, but these organs tend not to be examined during swine carcasses official inspection (Gortazar et al., 2011). Although, identification of specific members of MTBC is desired to provide proper treatment or control measures in STB. But it is quite difficult currently and time-consuming, and may produce false-negative results. Since swine appear to produce humoral responses soon after *M. bovis* infection, and these humoral responses are maintained during the development of disease, antibody diagnostic technique is suitable for STB prevention and control (Garrido et al., 2011; Roos et al., 2016). PPD-B-ELISA is an ideal choice to identify the risk of STB transmission due to its advantages of simple, easy, rapid, and high-throughput screening. In addition, the 6 positive sera and 20 randomly collected negative sera were tested with PPD-A-ELISA and the results were negative, which means PPD-B-ELISA had an excellent analytical specificity and the seropositive swine were infected with MTBC other than environmental mycobacteria.

To our knowledge, this is the first cross-sectional survey of STB in China. Our data indicated that the STB prevalence in Gansu province, China was 0.22% (95% CI: 0.04%, 0.63%), which was lower than that in Egypt (8.90%) (Mohamed et al., 2009), Ethiopia (5.83%) (Arega et al., 2013), Uganda 3.1% (Muwonge et al., 2010), and Spain (2.3%) (Cano-Terriza et al., 2018). STB prevalence in Gansu province, China was the same as that in Czech Republic (0.22%) which has been included amongst the states declared free from bovine tuberculosis within the European Union on February 31, 2004 (Pavlik et al., 2005). The lower STB prevalence in Gansu province might be associated with the preventive measures adopted by China since the Ministry of Agriculture and Rural Affairs of the People's Republic of China launched the control and eradication project on bovine TB in 2012 (Shufang et al., 2015). In June 2017, the Ministry of Agriculture and Rural Affairs of the People's Republic of China issued the National Guidelines for Controlling bovine TB (2017–2020), which adopted comprehensive prevention and control measures such as quarantine culling, risk assessment, movement control, and strengthening health management (The Ministry of Agriculture and Rural Affairs of the People's Republic of China., 2017). The World Organization for Animal Health, the World Health Organization, the Food and Agriculture Organization, and the International Union Against Tuberculosis and Lung Disease also jointly launched the first-ever roadmap to tackle zoonotic TB in 2017.

The STB prevalence in Guangxi province, China (1.67%, 95% CI: 0.35%, 4.79%) was significantly higher than that in Gansu province, China ($p < 0.05$) with OR of 7.77 (95% CI: 1.56, 38.81). The relatively higher bTB prevalence in Guangxi province might be due to the fact that the cold and humid environment in the winter in this region was more suitable for MTBC survival (Fine et al., 2011; Santos et al., 2015; Tanner et al., 1999). Wild or free-range swine usually exhibit higher STB prevalence than intensively raised swine. However, in this study, the STB prevalence in intensively raised pigs (0.23%, 95% CI: 0.05%, 0.68%) was higher than that in free-range Tibetan pigs (0.00%, 95% CI: 0.00%, 3.85%), but the difference between them was not significant ($p = 0.81$). The reason for this might be the limited serum samples of Tibetan pigs used in this study. Future investigations are suggested to collect more serum samples, especially from free-range Tibetan pigs to elucidate the epidemiological status of STB.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (31260616), Herbivorous Livestock Technology System in Gansu Province, and Integrated Experimental Demonstration Project of Disease Prevention and Control and Biosafety Technology for Healthy Pig Breeding (20201007-2). We thank all staff members from Gansu Agricultural University and Lanzhou Veterinary Research Institute who provided support for this study. Great gratitude goes to linguistics Prof. Ping Liu from Huazhong Agriculture University, Wuhan, China for her work at English editing and language polishing.

Conflict of Interest Statement

The authors declare no conflict of interest.

References

Amato, B., Lo Presti, V. D., Gerace, E., Capucchio, M. T., Vitale, M., Zanghi, P., . . . Boniotti, M. B. (2018). Molecular epidemiology of Mycobacterium tuberculosis complex strains isolated from livestock and wild animals in Italy suggests the need for a different eradication strategy for bovine tuberculosis. *Transboundary and Emerging Diseases, 65* (2), e416-e424. doi:10.1111/tbed.12776

Arega, S. M., Conraths, F. J., & Ameni, G. (2013). Prevalence of tuberculosis in pigs slaughtered at two abattoirs in Ethiopia and molecular characterization of Mycobacterium tuberculosis isolated from tuberculous-like lesions in pigs. *BMC Veterinary Research, 9* . doi:Artn 9710.1186/1746-6148-9-97

Bailey, S. S., Crawshaw, T. R., Smith, N. H., & Palgrave, C. J. (2013). Mycobacterium bovis infection in domestic pigs in Great Britain. *Veterinary Journal, 198* (2), 391-397. doi:10.1016/j.tvjl.2013.08.035

Bayraktar, B., Bulut, E., Baris, A. B., Toksoy, B., Dalgic, N., Celikkan, C., & Sevgi, D. (2011). Species Distribution of the Mycobacterium tuberculosis Complex in Clinical Isolates from 2007 to 2010 in Turkey:

a Prospective Study. *Journal of Clinical Microbiology, 49* (11), 3837-3841. doi:10.1128/Jcm.01172-11

Boadella, M., Lyashchenko, K., Greenwald, R., Esfandiari, J., Jaroso, R., Carta, T., . . . Gortazar, C. (2011). Serologic tests for detecting antibodies against Mycobacterium bovis and Mycobacterium avium subspecies paratuberculosis in Eurasian wild boar (Sus scrofa scrofa).*Journal of Veterinary Diagnostic Investigation, 23* (1), 77-83. doi:10.1177/104063871102300111

Bollo, E., Ferroglio, E., Dini, V., Mignone, W., Biolatti, B., & Rossi, L. (2000). Detection of Mycobacterium tuberculosis complex in lymph nodes of wild boar (Sus scrofa) by a target-amplified test system.*Journal of Veterinary Medicine. B: Infectious Diseases and Veterinary Public Health, 47* (5), 337-342. doi:10.1046/j.1439-0450.2000.00354.x

Buddle, B. M., Livingstone, P. G., & de Lisle, G. W. (2009). Advances in ante-mortem diagnosis of tuberculosis in cattle. *New Zealand Veterinary Journal, 57* (4), 173-180. doi:10.1080/00480169.2009.36899

Cano-Terriza, D., Risalde, M. A., Rodriguez-Hernandez, P., Napp, S., Fernandez-Morente, M., Moreno, I., . . . Garcia-Bocanegra, I. (2018). Epidemiological surveillance of Mycobacterium tuberculosis complex in extensively raised pigs in the south of Spain. *Preventive Veterinary Medicine, 159* , 87-91. doi:10.1016/j.prevetmed.2018.08.015

Corner, L. A. The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: how to assess the risk. (0378-1135 (Print)).

Corner, L. A. L., Gormley, E., & Pfeiffer, D. U. (2012). Primary isolation of Mycobacterium bovis from bovine tissues: Conditions for maximising the number of positive cultures. *Veterinary Microbiology, 156* (1-2), 162-171. doi:10.1016/j.vetmic.2011.10.016

Cousins, D. V., & Florisson, N. (2005). A review of tests available for use in the diagnosis of tuberculosis in non-bovine species. *Revue Scientifique Et Technique-Office International Des Epizooties, 24* (3), 1039-1059. doi:DOI 10.20506/rst.24.3.1635

Csivincsik, A., Ronai, Z., Nagy, G., Sveda, G., & Halasz, T. (2016). Surveillance of Mycobacterium caprae infection in a wild boar (Sus scrofa) population in south-western Hungary. *Veterinarski Arhiv, 86* (6), 767-775.

de Val Perez, B., Lopez-Soria, S., Nofrarias, M., Martin, M., Vordermeier, H. M., Villarreal-Ramos, B., . . . Domingo, M. (2011). Experimental model of tuberculosis in the domestic goat after endobronchial infection with Mycobacterium caprae. *Clinical and Vaccine Immunology, 18* (11), 1872-1881. doi:10.1128/CVI.05323-11

Di Marco, V., Mazzone, P., Capucchio, M. T., Boniotti, M. B., Aronica, V., Russo, M., . . . Marianelli, C. (2012). Epidemiological significance of the domestic black pig (Sus scrofa) in maintenance of bovine tuberculosis in Sicily. *Journal of Clinical Microbiology, 50* (4), 1209-1218. doi:10.1128/JCM.06544-11

Ereqat, S., Nasereddin, A., Azmi, K., Abdeen, Z., Greenblatt, C. L., Spigelman, M., . . . Bar-Gal, G. K. (2012). Genetic characterization of Mycobacterium tuberculosis in the West Bank, Palestinian Territories.*BMC Research Notes, 5* , 270. doi:10.1186/1756-0500-5-270

Fetene, T., Kebede, N., & Alem, G. (2011). Tuberculosis Infection in Animal and Human Populations in Three Districts of Western Gojam, Ethiopia. *Zoonoses and Public Health, 58* (1), 47-53. doi:10.1111/j.1863-2378.2009.01265.x

Fine, A. E., Bolin, C. A., Gardiner, J. C., & Kaneene, J. B. (2011). A Study of the Persistence of Mycobacterium bovis in the Environment under Natural Weather Conditions in Michigan, USA. *Veterinary Medicine International, 2011* , 765430. doi:10.4061/2011/765430

Garcia-Bocanegra, I., de Val, B. P., Arenas-Montes, A., Paniagua, J., Boadella, M., Gortazar, C., & Arenas, A. (2012). Seroprevalence and Risk Factors Associated to Mycobacterium bovis in Wild Artiodactyl Species from Southern Spain, 2006-2010. *PloS One, 7* (4). doi:ARTN e3490810.1371/journal.pone.0034908

Garcia-Jimenez, W. L., Salguero, F. J., Fernandez-Llario, P., Martinez, R., Risco, D., Gough, J., . . . Gomez, L. (2013). Immunopathology of granulomas produced by Mycobacterium bovis in naturally infected wild boar. *Veterinary Immunology and Immunopathology, 156* (1-2), 54-63. doi:10.1016/j.vetimm.2013.09.008

Garrido, J. M., Sevilla, I. A., Beltran-Beck, B., Minguijon, E., Ballesteros, C., Galindo, R. C., . . . Gortazar, C. (2011). Protection against tuberculosis in Eurasian wild boar vaccinated with heat-inactivated Mycobacterium bovis. *PloS One, 6* (9), e24905. doi:10.1371/journal.pone.0024905

Gil, O., Diaz, I., Vilaplana, C., Tapia, G., Diaz, J., Fort, M., . . . Cardona, P. J. (2010). Granuloma Encapsulation Is a Key Factor for Containing Tuberculosis Infection in Minipigs. *PloS One, 5* (3). doi:ARTN e1003010.1371/journal.pone.0010030

Gortazar, C., Vicente, J., Boadella, M., Ballesteros, C., Galindo, R. C., Garrido, J., . . . de la Fuente, J. (2011). Progress in the control of bovine tuberculosis in Spanish wildlife. *Veterinary Microbiology, 151* (1-2), 170-178. doi:10.1016/j.vetmic.2011.02.041

Hlokwe, T. M., van Helden, P., & Michel, A. L. Evidence of increasing intra and inter-species transmission of Mycobacterium bovis in South Africa: are we losing the battle? (1873-1716 (Electronic).

Kazwala, R. R., Daborn, C. J., Sharp, J. M., Kambarage, D. M., Jiwa, S. F., & Mbembati, N. A. (2001). Isolation of Mycobacterium bovis from human cases of cervical adenitis in Tanzania: a cause for concern?*International Journal of Tuberculosis and Lung Disease, 5* (1), 87-91.

Martin-Hernando, M. P., Hofle, U., Vicente, J., Ruiz-Fons, F., Vidal, D., Barral, M., . . . Gortazar, C. (2007). Lesions associated with Mycobacterium tuberculosis complex infection in the European wild boar.*Tuberculosis (Edinb), 87* (4), 360-367. doi:10.1016/j.tube.2007.02.003

Mayer, F. Q., Cerva, C., Driemeier, D., da Cruz, C. E. F., Loiko, M. R., Coppola, M. D., . . . Bertagnolli, A. C. (2012). Mycobacterium bovis infection in a collared peccary (Tayassu tajacu): Insights on tuberculosis wild reservoirs. *Veterinary Microbiology, 160* (3-4), 549-551. doi:10.1016/j.vetmic.2012.06.033

Mfinanga, S. G., Morkve, O., Kazwala, R. R., Cleaveland, S., Sharp, M. J., Kunda, J., & Nilsen, R. (2004). Mycobacterial adenitis: role of Mycobacterium bovis, non-tuberculous mycobacteria, HIV infection, and risk factors in Arusha, Tanzania. *East African Medical Journal, 81* (4), 171-178. doi:10.4314/eamj.v81i4.9150

Miller, M., Buss, P., de Klerk-Lorist, L. M., Hofmeyr, J., Hausler, G., Lyashchenko, K., . . . van Helden, P. (2016). Application of Rapid Serologic Tests for Detection of Mycobacterium bovis Infection in Free-Ranging Warthogs (Phacochoerus africanus)-Implications for Antemortem Disease Screening. *Journal of Wildlife Diseases, 52* (1), 180-182. doi:10.7589/2015-07-186

Mohamed, A. M., Abou El-Ella, G. A., & Nasr, E. A. (2009). Phenotypic and molecular typing of tuberculous and nontuberculous Mycobacterium species from slaughtered pigs in Egypt. *Journal of Veterinary Diagnostic Investigation, 21* (1), 48-52. doi:10.1177/104063870902100107

Muwonge, A., Kankya, C., Godfroid, J., Djonne, B., Opuda-Asibo, J., Biffa, D., . . . Skjerve, E. (2010). Prevalence and associated risk factors of mycobacterial infections in slaughter pigs from Mubende district in Uganda. *Tropical Animal Health and Production, 42* (5), 905-913. doi:10.1007/s11250-009-9506-5

Naranjo, V., Gortazar C Fau - Vicente, J., Vicente J Fau - de la Fuente, J., & de la Fuente, J. Evidence of the role of European wild boar as a reservoir of Mycobacterium tuberculosis complex. (0378-1135 (Print)).

Nugent, G., Gortazar, C., & Knowles, G. (2015). The epidemiology of Mycobacterium bovis in wild deer and feral pigs and their roles in the establishment and spread of bovine tuberculosis in New Zealand wildlife.*New Zealand Veterinary Journal, 63* , 54-67. doi:10.1080/00480169.2014.963792

Pavlik, I., Matlova, L., Dvorska, L., Shitaye, J. E., & Parmova, I. (2005). Mycobacterial infections in cattle and pigs caused by Mycobacterium avium complex members and atypical mycobacteria in the Czech

Republic during 2000- .... *Veterinarni Medicina, 50* (7), 281-290.

Pedersen, K., Miller, R. S., Anderson, T. D., Pabilonia, K. L., Lewis, J. R., Mihalco, R. L., . . . Gidlewski, T. (2017). Limited Antibody Evidence of Exposure To Mycobacterium Bovis in Feral Swine (Sus Scrofa) in the USA. *Journal of Wildlife Diseases, 53* (1), 30-36. doi:10.7589/2016-07-164

Perez de Val, B., Napp, S., Velarde, R., Lavin, S., Cervera, Z., Singh, M., . . . Mentaberre, G. (2017). Serological Follow-up of Tuberculosis in a Wild Boar Population in Contact with Infected Cattle. *Transboundary and Emerging Diseases, 64* (1), 275-283. doi:10.1111/tbed.12368

Roos, E. O., Buss, P., de Klerk-Lorist, L. M., Hewlett, J., Hausler, G. A., Rossouw, L., . . . Miller, M. A. (2016). Test performance of three serological assays for the detection of Mycobacterium bovis infection in common warthogs (Phacochoerus africanus). *Veterinary Immunology and Immunopathology, 182* , 79-84. doi:10.1016/j.vetimm.2016.10.006

Santos, N., Santos, C., Valente, T., Gortazar, C., Almeida, V., & Correia-Neves, M. (2015). Widespread Environmental Contamination with Mycobacterium tuberculosis Complex Revealed by a Molecular Detection Protocol. *PloS One, 10* (11). doi:ARTN e014207910.1371/journal.pone.0142079

Schiller, I., Oesch, B., Vordermeier, H. M., Palmer, M. V., Harris, B. N., Orloski, K. A., . . . Waters, W. R. (2010). Bovine Tuberculosis: A Review of Current and Emerging Diagnostic Techniques in View of their Relevance for Disease Control and Eradication. *Transboundary and Emerging Diseases, 57* (4), 205-220. doi:10.1111/j.1865-1682.2010.01148.x

Shitaye, J. E., Tsegaye, W., & Pavlik, I. (2007). Bovine tuberculosis infection in animal and human populations in Ethiopia: a review. *Veterinarni Medicina, 52* (8), 317-332. doi:10.17221/1872-Vetmed

Shufang Sun, Yuanyuan Wang, Lushi Liu, Yan Wang, Hongtao Sun, Rong Wei, Baoxu Huang. (2015). Analysis of Key Legal Points of Bovine Tuberculosis Eradication Plans in Developed Countries. *China Animal Health Inspection* , *32* (12), 41-44

Sun, L., Chen, Y., Yi, P., Yang, L., Yan, Y., Zhang, K., . . . Guo, A. (2021). Serological detection of Mycobacterium Tuberculosis complex infection in multiple hosts by One Universal ELISA. *PloS One, 16* (10), e0257920. doi:10.1371/journal.pone.0257920

Tanner, M., & Michel, A. L. (1999). Investigation of the viability of M-bovis under different environmental conditions in the Kruger National Park. *Onderstepoort Journal of Veterinary Research, 66* (3), 185-190.

Tbran F, Popa R, Marica R, Toma C. (2019). Tuberculosis in pigs: a review of the current status. *Porcine Researsch* , *9* , 39-49.

Table 1 Cross-sectional study of STB in Gansu province, China

City	Total swine Population (million)	Calculated sample size	Individual level	Individual level	Individual level	Herd level	Herd level	Herd le
			No. of positive	No. of tested	Prevalence% (95% CI: lower, upper)	No. of positive	No. of tested	Prevale (95% C lower, upper)
Lanzhou	0.43	41.44	0	90	0.00 (0.00, 4.02)	0	3	0.00 (0. 70.76)
Jiayuguan	0.04	3.85	0	150	0.00 (0.00, 2.43)	0	5	0.00 (0. 52.18)
Jinchang	0.05	4.82	0	90	0.00 (0.00, 4.02)	0	3	0.00 (0. 70.76)

City	Total swine Population (million)	Calculated sample size	Individual level	Individual level	Individual level	Herd level	Herd level	Herd le
Baiyin	0.84	80.95	0	90	0.00 (0.00, 4.02)	0	3	0.00 (0. 70.76)
Tianshui	0.65	62.64	0	96	0.00 (0.00, 3.77)	0	3	0.00 (0. 70.76)
Wuwei	1.49	143.59	1	90	1.11 (0.03, 6.04)	1	3	33.33 (0 90.57)
Zhangye	0.65	62.64	2	91	2.20 (0.27, 7.71)	1	3	33.33 (0 90.57)
Pingliang	0.44	42.40	0	100	0.00 (0.00, 3.62)	0	3	0.00 (0. 70.76)
Jiuquan	0.27	26.02	0	122	0.00 (0.00, 2.98)	0	4	0.00 (0. 60.24)
Qingyang	0.46	44.33	0	90	0.00 (0.00, 4.02)	0	3	0.00 (0. 70.76)
Dingxi	0.52	50.11	0	90	0.00 (0.00, 4.02)	0	3	0.00 (0. 70.76)
Longnan	0.61	58.79	0	90	0.00 (0.00, 4.02)	0	3	0.00 (0. 70.76)
Linxia	0.21	20.24	0	96	0.00 (0.00, 3.77)	0	3	0.00 (0. 70.76)
Gannan	0.23	22.17	0	94	0.00 (0.00, 3.85)	0	3	0.00 (0. 70.76)
In total	6.89	664	3	1379	0.22 (0.04, 0.63)	2	45	4.44 (0. 15.15)

FIGURE 1. Gansu province in the Chinese map

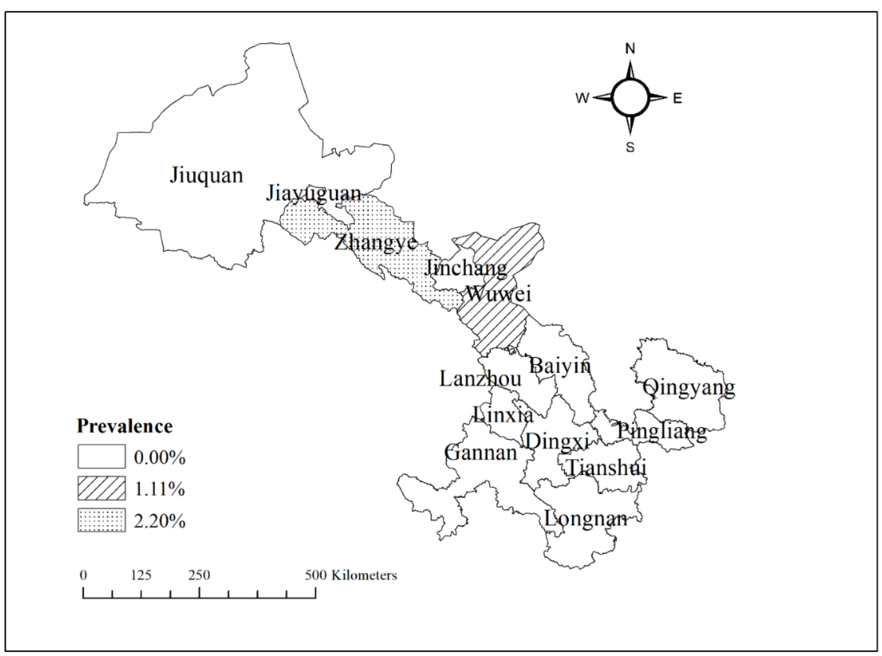

FIGURE 2. STB prevalence in Gansu province, China at individual level