Development of evidence-based strategies to control *Brucella* spp. in dairy herds in Henan Province, China

A thesis presented in partial fulfilment of the requirements for the degree of

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Yu Wang

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Abstract

Brucella spp. remains a significant challenge in China, affecting the dairy industry and public health despite decades of voluntary control measures. This thesis aims to address key knowledge gaps in diagnosis, epidemiology, and disease impact of *Brucella* spp. in dairy herds to inform evidence-based control strategies in Henan Province, China.

A scoping review of 61 studies (2004-2022) characterized the epidemiological landscape, revealing that *B. abortus* biovar 3 predominated 85.8% of *Brucella* spp. isolates recovered from dairy cattle in China. Considerable heterogeneity was observed in prevalence estimates and *Brucella* spp. isolation across provinces. These findings guided subsequent investigations into diagnostic accuracy, biosecurity practices, disease impact, and financial analyses.

To enhance diagnostic accuracy, a cross-sectional study evaluated the diagnostic performance of four serological tests in Henan dairy herds. Using a novel Bayesian latent class model, optimal cut-off values were established for fluorescence polarization assay and competitive ELISA, estimating test sensitivity (69.7%-89.9%) and specificity (97.1%-99.6%). These findings provide a foundation for improving brucellosis diagnostic strategies.

Beyond diagnosis, an assessment of farm biosecurity practices and stakeholder motivations was conducted using a structured questionnaire. Two distinct clusters of dairy herds were identified. Cluster 1, characterized by medium (400-1000) to large (>1000) herd sizes and higher educational levels, showed better adherence to proper biosecurity practices than Cluster 2. Stakeholders prioritized disease impacts and economic losses, highlighting the need for integrating these into the brucellosis control programs.

To address this knowledge gap about disease impact, a longitudinal study estimated the effects of *Brucella* seroconversion on key production indicators. The annual incidence of seroconversion of brucellosis was 13.1% (95% CI: 10.9, 15.6) at the cow level. Seroconverted cows exhibited a reduction in daily milk yield (3.2 kg/day, 95% CI: 2.4, 4.0), elevated somatic cell counts, and increased pregnancy loss (relative risk: 4.26, 95% CI: 3.17, 5.73), compared to that of consistently negative cows. These findings provided essential epidemiological insights into the seroconversion of *Brucella* spp. and its implications on three essential dairy productivity outcomes.

Building on these epidemiological insights, a financial analysis estimated the direct annual costs of *Brucella* infection at 78.9 Chinese Yuan (CNY) per animal and 4,019 CNY per infected cow. Among the three evaluated interventions (vaccination, test-and-culling, and test-and-culling plus vaccination), vaccination was the most cost-effective, yielding a benefit-cost ratio of 5.84 (95% CI: 4.34 – 7.42) and a net present value of 408.2 thousand CNY over ten years.

Overall, this thesis integrates epidemiological, diagnostic, biosecurity, and financial analyses to inform evidence-based brucellosis control in Henan dairy herds. These findings could support farm stakeholders and policymakers in improving the control of *Brucella* spp. in Henan dairy herds, contributing to broader brucellosis control efforts in China.

Acknowledgments

Writing the acknowledgment section of my thesis is both the most difficult and the most meaningful part for me. Although I write these words in plain English without assistance from others, they still cannot fully convey the depth of my gratitude to all those who helped and contributed to this thesis.

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List of Publications

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Chapter 1 Introduction

1.1 Background

The dairy industry provides high-quality food sources and facilitates sustainable socio-economic development. Dairy farmers continually strive to enhance their herd productivity and management efficiency to achieve commercial success in an increasingly competitive market. In China, recent efforts have been dedicated to controlling zoonotic diseases that threaten animal productivity, welfare, and public health. Among these, brucellosis has re-emerged as a critical challenge for both China's dairy sector and public health (Wang et al., 2021b).

Brucellosis is a highly contagious bacterial disease with a range of susceptible hosts, including domestic animals, wildlife, and humans. The primary causative agent of brucellosis in dairy cattle is *Brucella abortus* (*B. abortus*), a species of the *Brucella* genus within the family *Brucella*ceae (Whatmore et al., 2021). The annual incidence of notifications in humans increased from 2.11 per 100,000 in 2004 to 5.06 per 100,000 in 2022 (Wang et al., 2024b). Recent events, such as the Lanzhou *Brucella* vaccine production leak (Pappas, 2022), have raised public awareness and concern about the disease (Lai et al., 2017). Furthermore, a meta-analysis reported a substantial increase in the animal-level seroprevalence of brucellosis in Chinese dairy cattle from 1.6% in 2008–2012 to 2.6% in 2013–2018 (Ran et al., 2019). These alarming increases in both livestock and humans necessitate a more effective control strategy to combat brucellosis in China.

While lessons from successful elimination programs in countries such as Australia and New

Zealand offer valuable insights and directions (Zhang et al., 2018), their direct application in China is impractical due to differences in farm demographics, available resources, farm biosecurity, animal movement management, and infrastructure. Before the start of the thesis, the primary strategy adopted for brucellosis in dairy cattle in China was test-and-culling with a ban on vaccination, complemented by farm biosecurity and animal movement control (Ministry of Agriculture and Rural Affairs, 2016). However, knowledge gaps that tamper with the effectiveness of brucellosis control programs in dairy cattle have not been sufficiently studied in China. In particular, existing control strategies have not been sufficiently tailored to the unique characteristics of Chinese dairy herds and motivations among farm stakeholders. Compared to brucellosis in small ruminants and humans, brucellosis in dairy cattle has received limited research attention. Key research questions remain unresolved, including:

1. Which diagnostic strategy should be chosen for the specific epidemiological scenario in China?
2. How do current diagnostic tests perform in Chinese dairy populations?
3. How much does brucellosis affect production?
4. Which control strategy (vaccination or test-and-culling) is more financially viable for Chinese dairy herds?

Addressing these questions is essential for developing evidence-based strategies to guide brucellosis control in China’s dairy industry.

1.2 Dairy Industry in China and Henan Province

The research presented in the thesis focused on the dairy cattle industry in Henan Province, one of the major milk production provinces in China. China's dairy industry has developed rapidly in the past decade. The total national milk yield increased from 30.4 million tons in 2017 to 36.8 million tons in 2021, while the number of dairy cattle remained approximately 10.5 million during the same period (National Bureau of Statistics of China, 2023). Concurrently, China imported 1.04 million tons of liquid milk and 2.35 million tons of solid dairy products (including milk powder, butter, and whey powder) in 2020 (China International Import Expo Exhibitors Alliance Dairy Committee, 2021). Based on the assumption that one kg of solids equals eight kg of liquid milk, China imported a total of 19.8 million tons of fluid milk, with a ratio of 0.58 to domestically produced milk. These statistics imply that China faces a large gap in milk production and is highly dependent on dairy product imports.

Despite rising production, per capita dairy consumption in China remained comparatively low. Between 2017 and 2021, annual per capita dairy product consumption increased from 21.7 kg to 26.1 kg (National Bureau of Statistics of China, 2023), yet this figure remained well below the levels observed in developed countries, such as 231 kg/year in the United States of America and 222 kg/year in Australia, and the world average (87 kg/year) (FAO, 2023). These statistics indicate that China has substantial consumption potential for dairy products, motivating China to improve its dairy milk industry. According to the China Dairy Industry Report, dairy herds with a herd size of 200-499 animals accounted for 32.7% of the total, followed by farms with 500-1000 animals at 27.4% (China Dairy Association, 2022). Also, China is shifting toward larger, intensively managed dairy herds, replacing traditional free-ranging dairy herds. While this transition supports increased milk production, it also presents greater challenges to disease control and prevention. In 2020, the average per-cow milk production was reported to be 8.3 tons per lactation, showing an average annual growth rate of 5% since 2008.

Chinese dairy farms employ intensive barn feeding management based on crop silage and total mixed ration (TMR). Cattle are generally housed in independent cowsheds where they can move freely. The calving pattern is year-round, with a seasonal peak in winter and reduced calving in summer. Many dairy farms use synchronized estrus and fixed-time insemination programs to enhance conception probability (Guo et al., 2020), improving herd management efficiency and productivity. This calving pattern aligns with the seasonality of milk demand, which is lower in summer and peaks in winter.

In central China, Henan Province is one of China's major dairy milk-producing provinces, with 385 thousand dairy cattle producing 2.37 million tons of milk in 2023 (National Bureau of Statistics of China, 2023). However, there are still no publicly accessible statistics on the spatial distribution and demographic information of Henan dairy herds, making understanding the production and management of dairy herds across the entire province challenging. An alternative solution is to assess the demographic and production information through a collaboration with Henan Dairy Herd Improvement Centre, which has been involved in monitoring milk production and components. A map showing the distribution of dairy herds in Henan Province is plotted in Figure 1.1. Beef farms were excluded from the scope of this thesis because of their more complex husbandry patterns and unknown farming characteristics compared to dairy herds in China. From a policy perspective, Henan province has been improving the competitiveness of its dairy industry through a range of approaches in recent years, including genetic variety improvement, animal husbandry production management, and disease control (General Office of Henan Provincial Government, 2022). Brucellosis is one of the diseases that are given priority control in dairy cattle in Henan province because of its significant impact on dairy cow production, food security, and public health (Department of Agriculture and Rural Affairs of Henan Province, 2022a). The following sections will present specific issues regarding brucellosis in dairy cattle in Henan province and China.

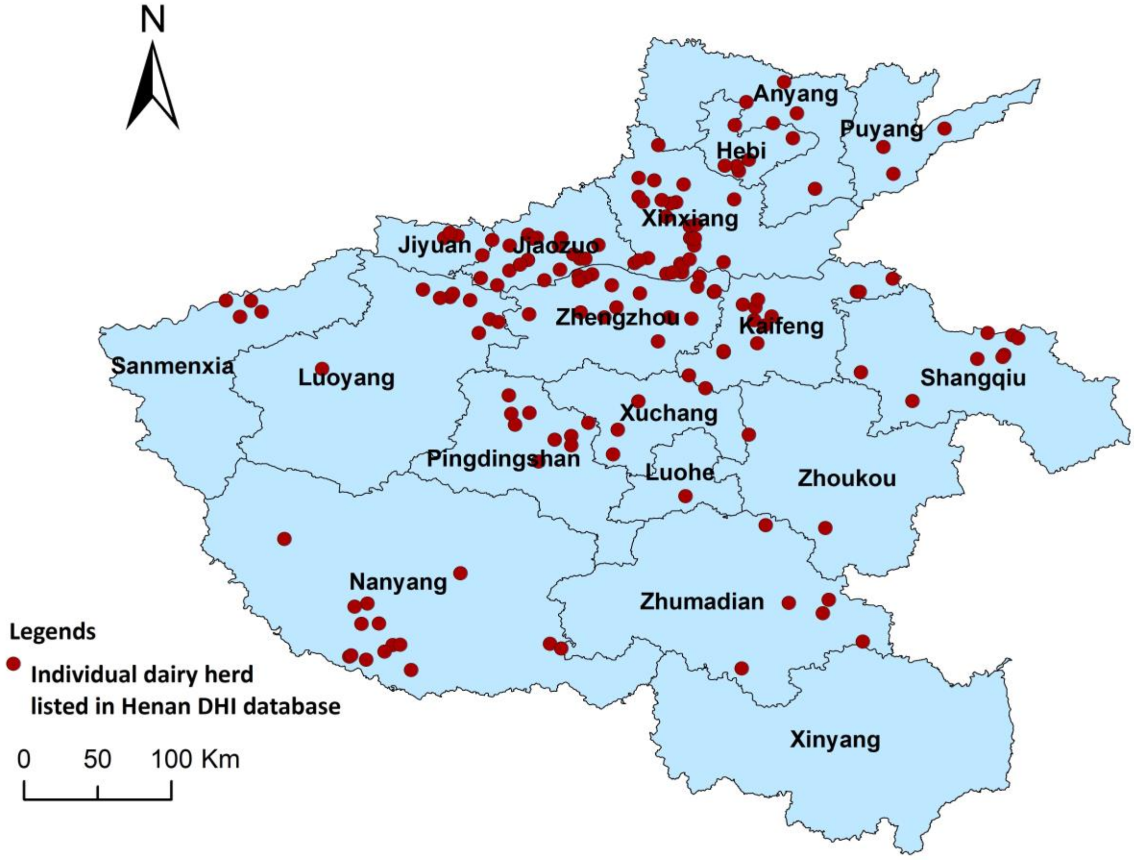


Figure 1. 1 A map showing the distribution of individual dairy herds listed in Henan Dairy Herd Improvement (DHI) Centre database.

1.3 Specific issues related to *Brucella* spp.

Given the substantial dairy cattle population in China and the severe consequences of *Brucella* spp. infection, China needs a more effective control program to mitigate its impact on dairy herds. Developing a sound *Brucella* control program requires a comprehensive understanding of multifaceted factors, including the epidemiology of *Brucella* spp., farm practices, diagnostic capabilities, and socioeconomic considerations. Integrating these diverse sources of evidence can guide stakeholders in designing and adopting feasible and cost-effective control measures. The following sections examine key issues related to *Brucella* control in the Chinese dairy industry.

1.3.1 Diagnostic tests

Diagnostic tests are crucial for confirming or screening the infection or exposure status at the animal, herd, and region levels, estimating disease prevalence, and demonstrating disease freedom (Greiner et al., 2000a). However, diagnostic tests are subject to inherent errors, including false positive results, which lead to the culling of healthy animals, and false negative results, which allow positive animals to remain on the farm in a test-and-slaughter program for brucellosis (Greiner et al., 2000a). The selection of cut-off values and estimation of diagnostic sensitivity and specificity are essential before applying diagnostic tests in clinical settings and epidemiological studies (Greiner et al., 2000c). Culture-positive or test-based positive samples are often used to calibrate cut-off values for continuous assays (e.g., ELISA) and estimate the sensitivity and specificity of diagnostic tests for *Brucella* spp. in China (Dong et al., 2021). Nonetheless, using culture as a gold reference test likely overestimates sensitivity and underestimates specificity because it fails to account for the existence of weakly positive samples (Greiner et al., 2000b). In the National Standard Diagnostic Techniques for Animal Brucellosis (GB/T 18646-2018), serial testing is recommended but potential conditional dependence is not considered. These knowledge gaps underscore the need for an accurate evaluation of diagnostic tests against *Brucella* spp. to provide robust benchmarks for implementing *Brucella* spp. control programs in China.

1.3.2 Epidemiology and disease impact of *Brucella* spp.

Disease incidence risk is an essential metric in epidemiology, representing the risk at which a disease spreads within a population over a specific period. Wang et al. (2020c) conducted a study estimating the overall animal-level incidence risk of brucellosis at 8.3% per three months (95% CI: 6.2, 10.8) across 15 dairy farms from Hubei province, varying from 0.0% to 90.9% over three months across the studied farms. However, incidence is influenced by various factors, such as biological attributes, population demographics, population immune status, environmental conditions, and interventions. Consequently, the incidence is population- and context-specific within a specific population over a particular period and should be re-estimated for different populations and periods to account for these influencing factors.

Understanding the impact of *Brucella* spp. on dairy cow productivity could help stakeholders prioritize disease control efforts. However, it is interesting that parameters used for disease impact were derived from expert opinions or assumed values. Bernués et al. (1997) suggested commonly used risk of abortions (15%) and milk losses (15%) for infected animals, even though these values were not empirically estimated. Nonetheless, these values have been widely applied in subsequent brucellosis economic analysis studies (Zeng et al., 2019b), as they offer plausible pictures for the impact of *Brucella* spp. on reproduction and production performance within dairy farms (Ferreira et al., 2023). Nevertheless, there is still a need to estimate the accurate disease impact based on empirical data to improve the understanding of *Brucella* spp. Desirable studies should also account for various factors particular to the unique context of the Chinese dairy industry, including management type, herd size, herd demographics, and other relevant variables. Accurate assessment of population-specific *Brucella* spp. impacts can motivate interest and attention among farm stakeholders and inform effective and targeted control measures to improve dairy farm productivity and financial vitality.

1.3.3 Farm biosecurity practices

Modern veterinary practice emphasizes disease prevention at the population level in addition to treating individual animals, focusing more on stringent farm biosecurity. Sound farm biosecurity measures encompass a series of management practices aimed at preventing the transmission of infectious agents or carriers between animal groups and preventing the introduction or escape of contagious agents from farms (Robertson, 2020). Robust biosecurity is essential to maintaining disease-free status for farms, regions, and countries while reducing the spread of infectious agents in disease-endemic farms and areas. Despite the widely recognized effectiveness of biosecurity measures among academics, implementing biosecurity on farms is voluntarily driven by understanding biosecurity, availability of resources, and motivations among farm stakeholders. Currently, there is limited knowledge about the adoption of appropriate farm biosecurity practices in dairy herds in China (Chen et al., 2021). Several biosecurity measures, such as hygienic measures and quarantine of introduced animals, have been demonstrated to be effective in preventing the transmission of zoonotic diseases from animals to humans (Youssef et al., 2021). Robertson (2020) reviewed good farm biosecurity practices and identified possible pathways of risk introduction and export (e.g., the introduction of contaminant via visitors, vehicles, and feed), and proposed feasible biosecurity mitigation solutions to build robust biosecurity defense at a farm, regional, and national level. Investigating the current status of biosecurity measures and identifying the determinants influencing their adoption is crucial for *Brucella* spp. control in China. Such information could provide valuable insights into the challenges faced by Chinese dairy farmers and guide the development of targeted educational and incentive programs, and ultimately, a more comprehensive and coordinated approach to *Brucella* spp. control for China's dairy industry.

1.3.4 Economic Considerations of *Brucella* spp.

Brucella spp. imposes a significant economic burden on dairy farms, with costs divided into direct and indirect losses (Montiel et al., 2015). Direct costs arise directly from new infections or cases of the disease, such as milk losses, increased abortion rate, lower fertility, treatment costs, and potential human infections (Montiel et al., 2015). Indirect costs resulted from long-term effects attributed to the disease, such as increased disease management costs, premature culling, replacement heifer purchase, trade limitations, and reduced genetic gain (Rushton et al., 1999). However, economic assessments are heavily driven by various parameters and model assumptions (Kiiza et al., 2023). Using population-specific parameters and assumptions is crucial for acquiring accurate economic estimates, as imprecise or irrelevant data can introduce significant bias. In the Chinese dairy industry context, there is currently no economic assessment study about *Brucella* spp. and control interventions in dairy cows, which likely undermines stakeholders’ priorities and enthusiasm for control efforts. Conducting an accurate economic analysis by using population-specific epidemiological and production data is essential to identify the most cost-effective control strategy and justify the economic returns of different interventions. Such economic analysis can help farm stakeholders implement measures on their herds.

1.3.5 One Health Perspective

Brucella spp. represents a One Health concern involving animals, humans, and the environment. The concept of One Health has been broadly recognized as moving away from focusing on the health of separate groups to the integral health of an ecosystem consisting of domestic and wild animals, humans, plants, and the wider environment (One Health High-Level Expert et al., 2022). The One Health approach recommends enhanced cooperation and collaboration between different health sectors in an equitable, multicultural, and transdisciplinarity manner at different levels (One Health High-Level Expert et al., 2022), beneficial to all health sectors (Zinsstag et al., 2007). Currently, the One Health approach is gaining popularity in the veterinary and medical communities in China, but challenges remain in coordination and collaboration among multiple departments and stakeholders. These challenges include defining the responsibilities and benefits for each stakeholder involved in the One Health approach, fostering multi-stakeholder collaboration, and ensuring transparent and accurate communication and information access. In the discussion of this thesis, the roles of farmers, researchers, and governments will be discussed within a One Health framework, combined with the findings presented in this thesis.

1.4 Thesis aims and structure

The primary objective of this thesis is to address the diagnostic, epidemiological, and economic knowledge gaps about *Brucella* spp. infection in China's dairy industry to provide scientific evidence and economic justification for implementing *Brucella* spp. control programs. To achieve this aim, studies were designed and conducted with the following objectives:

1) Describe the current epidemiology of *Brucella* infection in dairy cattle and humans, including prevalence/incidence, *Brucella* typing, and risk factors, through a literature review
2) Evaluate the diagnostic sensitivity and specificity, and optimal cut-off values of four serological tests against *Brucella* in Henan dairy herds;
3) Investigate farm biosecurity practices and motivations of dairy herd stakeholders regarding Brucella control programs;
4) Estimate the seroconversion risk of Brucella and its effects on milk yield, somatic cell count, and pregnancy;
5) Evaluate the financial losses of *Brucella*, and the costs and benefits of vaccination, test-and-culling, and vaccination plus test-and-culling programs to a dairy farm in Henan Province.

The thesis is structured into one chapter of a literature review and four subsequent studies, contributing to an insightful understanding of the disease and its control measures. Chapters 2 and 3 have been published in peer-reviewed journals at the time of submission of this thesis.

Chapter 2 presents a scoping review of published literature on the epidemiology of *Brucella* spp. in dairy cattle and humans in China between 2004 and 2022. The current epidemiological understanding of *Brucella* and studies on prevalence estimation and *Brucella* typing were summarized. The risk factors and existing knowledge gaps to *Brucella* control in dairy herds in China were also reviewed (Research Objective 1). This chapter has been published in the One Health.

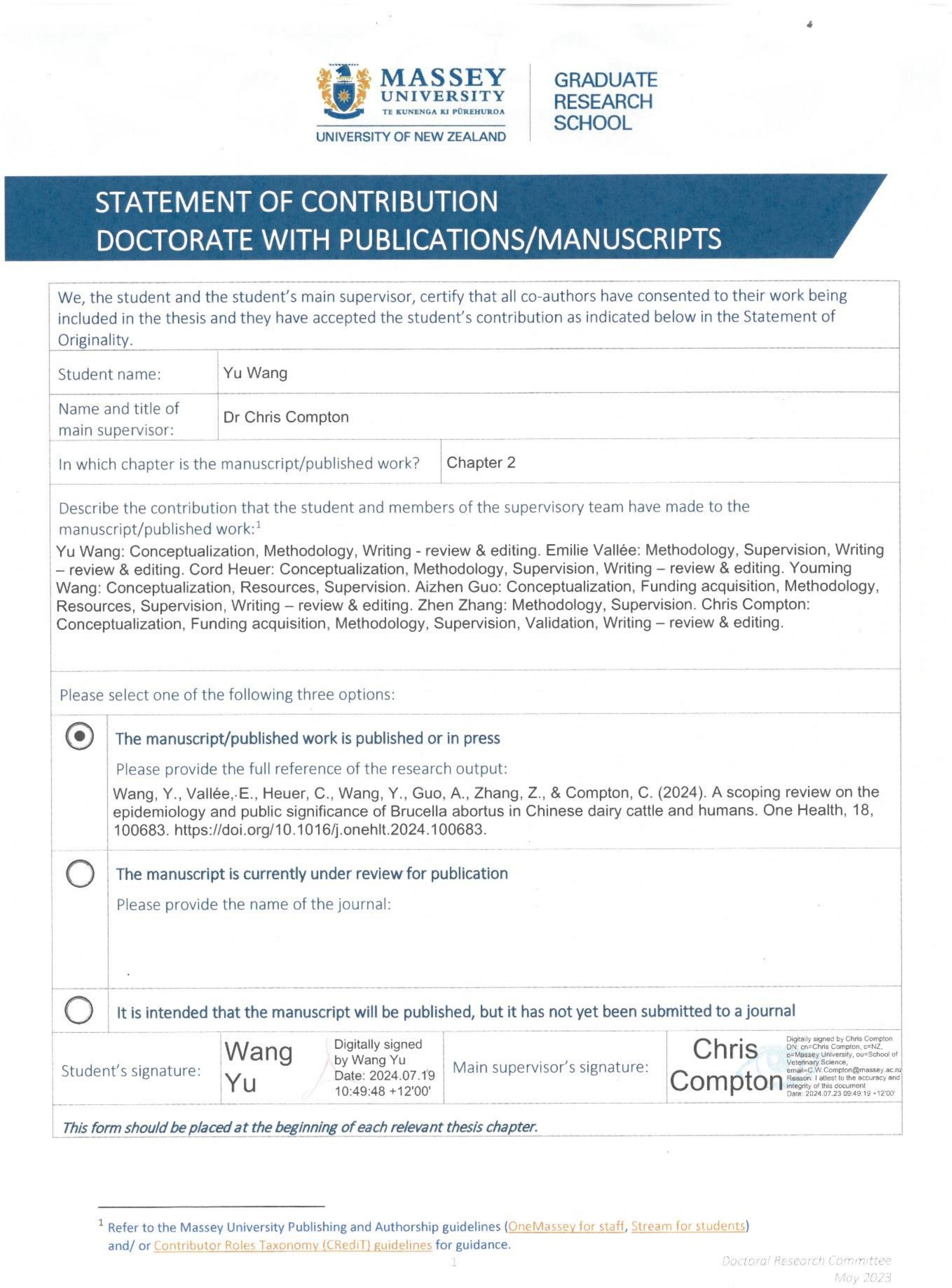
Chapter 3 developed a novel Bayesian latent class model to optimize cut-off values for two continuous tests and estimate the sensitivity and specificity of four serological tests used in the diagnosis of *Brucella* in dairy cattle. The findings provide essential benchmarks for accurately classifying *Brucella* spp. exposure status for individual animals (Research Objective 2). This chapter has been published in Preventive Veterinary Medicine.

Chapter 4 used a questionnaire survey to investigate several farm biosecurity practices and the motivations among selected Henan dairy herds regarding bovine brucellosis control programs. Understanding the current uptake of farm biosecurity practices and their determinants can effectively guide future targeted training programs to aid brucellosis control (Research Objective 3). This chapter is intended for publication in a peer-reviewed journal.

Assessing the impact of *Brucella* on animal productivity would help farm stakeholders and policymakers determine the priority of brucellosis versus competing diseases. In Chapter 5, serological data for *Brucella* were collected to estimate the incidence of seroconversion over one year from twelve dairy herds in Henan province, China. Production data was also collected to estimate the association between the serological status of *Brucella* and production outcomes. The results could provide valuable epidemiology information and disease impact on *Brucella* in dairy cows (Research Objective 4). This chapter is intended for publication in a peer-reviewed journal.

Chapter 6 presents a financial analysis for one baseline model and three feasible interventions for a typical Henan dairy herd. This analysis integrates findings and data collected from previous chapters to produce a cost-benefit analysis over a ten-year time horizon. Sensitivity analysis using alternative parameters and assumptions demonstrated the robustness of the results. This chapter is intended for publication in a peer-reviewed journal.

Chapter 7 discusses the implications of the main findings of the thesis and the contributions and benefits that various stakeholders can make from a One Health perspective. Study limitations are also discussed in Chapter 7. Given the novel knowledge and epidemiological information in this thesis, suggestions for future studies are proposed to address remaining knowledge gaps and improve the effectiveness of *Brucella* control programs in China.



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Student name:	Yu Wang
Name and title of main supervisor:	Dr Chris Compton
In which chapter is the manuscript/published work?	Chapter 2

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Yu Wang: Conceptualization, Methodology, Writing - review & editing. Emilie Vallée: Methodology, Supervision, Writing – review & editing. Cord Heuer: Conceptualization, Methodology, Supervision, Writing – review & editing. Youming Wang: Conceptualization, Resources, Supervision. Aizhen Guo: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. Zhen Zhang: Methodology, Supervision. Chris Compton: Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing.

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Doctoral Research Committee
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Chapter 2 A scoping review on the epidemiology and public significance of *Brucella* spp. in Chinese dairy cattle and humans

Yu Wang[a], Emilie Vallée[a], Cord Heuer[a], Youming Wang[b], Aizhen Guo[c], Zhen Zhang[d], Chris Compton[a]

[a] EpiCentre, School of Veterinary Science, Massey University, Private Bag 11-222, Palmerston North, New Zealand

[b] China Animal Health and Epidemiology Center, Qingdao, China

[c] National Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China

[d] Henan Dairy Herd Improvement Center, Zhengzhou, Henan, 450045, China

2.1 Abstract

Brucellosis, caused by *Brucella* spp., is a re-emerging One Health disease with increased prevalence and incidence in Chinese dairy cattle and humans, severely affecting animal productivity and public health. In dairy cattle, *Brucella abortus* is considered the primary causative agent, although infections with other *Brucella* species occur occasionally. However, the epidemiological and comparative importance of *B. abortus* in dairy cattle and humans remains inadequately understood throughout China due to the heterogeneity in locations, study designs, and sampling population. This scoping review aims to describe the changing status of

Brucella spp. infection in dairy cattle and humans, investigate the circulating *Brucella* species and biovars, and identify factors driving the disease transmission by retrieving publicly accessible literature from four databases. After passing the prespecified inclusion criteria, sixty original articles were included in the final synthesis. Although the reported animal-level and farm-level prevalence of brucellosis in dairy cattle was lower compared to other endemic countries (e.g., Iran and India), it has been reported to increase over the last decade. The annual incidence of notifications in humans increased from 2.11 per 100,000 in 2004 to 5.06 per 100,000 in 2022. The Rose Bengal Test and Serum Agglutination Test, interpreted in series, were the most used serological tests to diagnose *Brucella* spp. in dairy cattle and humans. *B. abortus* biovar 3 was the predominant species (81.9%) and biovar (85.8%) among all *Brucella* species isolated from dairy cattle, while 63.8% of *Brucella* spp. isolated from humans were *B. melitensis* biovar 3 in China. These *Brucella* spp. collected from humans and dairy cattle were mainly clustered in Inner Mongolia and Shannxi Province (75.7%), limiting the generalizability of the results to other provinces. Live cattle movement or trade was identified as the critical factor driving brucellosis transmission, but its movement pattern remains unknown within the Chinese dairy sector. These knowledge gaps highlight the need for a One Health approach to build close collaboration among multiple sectors and stakeholders in tackling *Brucella*. A coordinated and evidence-based research program is essential to inform regional or national control strategies that are feasible and economical in the Chinese context.

Keywords: dairy cattle, *Brucella abortus*, diagnosis, epidemiology, One Health, control strategy

2.2 Introduction

Brucellosis, caused by *Brucella* species *(Brucella* spp.*)*, is globally recognized as a significant zoonotic and One Health disease (Seleem et al., 2010). *Brucella* spp. contains more than twelve species of intracellular bacteria; among them, four zoonotic species infect multiple hosts, such as cattle and small ruminants (Whatmore et al., 2021). *Brucella* spp. exhibits host tropism but is not restricted to an exclusive host; *B. abortus* mainly infects cattle, whereas *B. melitensis* primarily affects small ruminants (Whatmore et al., 2021). Each *Brucella* species may have multiple biovars (subtypes) with different pathogenicity, host range, and geographical distribution (WOAH, 2022b). Characteristic clinical signs of *Brucella* spp. infection in cattle and small ruminants are abortion, retained placenta, orchitis, infertility, and reduced milk yield (Bernués et al., 1997; Dean et al., 2012b; Seleem et al., 2010). In humans, the symptoms of this disease include muscle pain, arthritis, rising and falling “undulant” fever, hyperhidrosis, fatigue, and night sweats (Dean et al., 2012a). Given the profound impact of *B. abortus* on humans and livestock, the World Organisation for Animal Health (WOAH, founded as OIE) and many countries have classified *B. abortus* as a notifiable or listed disease affecting the international trade in animals and their products (https://www.woah.org/en/disease/brucellosis/).

Brucella spp. is a highly contagious pathogen that can spread across multiple hosts. The primary transmission routes of *Brucella* spp. in cattle are by direct contact with aborted products or vaginal secretions of infected animals or consumption of unpasteurized milk (Dadar et al., 2020). Contacting wild animals (e.g., rodents and deer) and ticks may also provoke the re-emergence of *Brucella* spp. infection in domestic animals (Godfroid et al., 2013; Kamath et al., 2016; Zheludkov et al., 2010). Direct contact with infected animals and their aborted products without wearing personal protection equipment (PPE, e.g., gloves and masks) is considered one of the riskiest ways to acquire brucellosis in humans (Franco et al., 2007; Wang et al., 2021b). Consumption of raw or unpasteurized dairy products and inhaling *Brucella* aerosols are common routes of *Brucella* spp acquisition in humans (Baoshan et al., 2021; Kiambi et al., 2020; Pappas, 2022; Seleem et al., 2010). Therefore, *Brucella* spp. is regarded as a One Health hazard to animals, occupation-related workers, and the public.

Since almost all brucellosis in humans is of animal origin, prevention of brucellosis in humans could be achieved through either human hygiene, food safety measures, or control measures in infected animal populations. Controlling *Brucella* on farms is an efficient and cost-effective approach that also reduces the burden of animal disease and lowers the risk of spillover to humans (Roth et al., 2003; Singh et al., 2018; Zeng et al., 2019b). Intensified surveillance in animal host species, test-and-slaughter, restriction of live animal movement, and mass vaccination are effective brucellosis control strategies (Zhang et al., 2018). Australia and New Zealand have successfully eliminated *B. abortus* from domestic farms by implementing extensive mandatory vaccination, followed by a rigorous test-and-culling approach, strict farm biosecurity measures, and animal movement control (Adlam, 1978; Davidson, 2002). In areas where *Brucella* spp. is endemic, vaccinating susceptible animals is highly recommended by WOAH to provide protection against virulent *Brucella* spp. and mitigate disease impacts on reproduction and production (WOAH, 2023b).

Brucella spp. is still endemic in many countries, with a particularly high prevalence in Africa and Asia, including China (Laine et al., 2023; Pappas et al., 2006). Given China's substantial population of 3.09 million dairy cows, addressing this production-limiting and zoonotic disease at the population level is crucial for economics, food security, and One Health (Peng et al., 2020b). However, *B. melitensis* is widely considered a primary concern in humans (Deqiu et al., 2002; Lai et al., 2017), indirectly resulting in *B. abortus* being overlooked in dairy cows and humans for a long time. There is still controversy over the relative importance of *B. abortus* to *B. melitensis* in cows and humans and whether to adopt more intense control measures in cows in China. A comprehensive understanding of the current epidemiology of *Brucella* spp. in dairy cattle and humans is a prerequisite for making evidence-based decisions about this pathogen and facilitating the comparative opportunities in facilitating brucellosis One Health management. However, existing literature reports are heterogeneous in geographical location, study design, and reporting quality, making it difficult to assess the overall *Brucella* spp. status in China. A scoping review is a type of literature review that transparently and reproducibly integrates the current literature on a related topic with little or no research done and can also be a precursor to a subsequent systematic review (Munn et al., 2018). Although many works of literature report brucellosis in China, there has not been a formal scoping review on *Brucella* spp. in dairy cows and humans. This scoping review aimed to: (1) provide an overview of *Brucella* spp. status in Chinese dairy cattle and human populations between 1st January 2004 and 31st December 2022; and (2) identify the key factors driving the spread of *Brucella* spp. The insights gleaned from this review can be used to tailor evidence-based control strategies to reduce the disease burden both in dairy farms and humans in China.

2.3 Materials and Methods

2.3.1 Literature search strategy

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009), a comprehensive literature search was conducted to identify scientific articles published from 1st January 2004 to 31st December 2022 using four databases (PubMed, Web of Science, Scopus, China National Knowledge Infrastructure - CNKI) as shown in Figure 2.1. The year 2004 was chosen as the starting point for the search because the Chinese government upgraded the national infectious disease notification system in 2004 (Lai et al., 2017) and therefore previous notification data would not be comparable to data reported from 2004 onward. A complete list of the search terms and their combinations used for each database is available in Table S 1.1 of Appendix 1. The reference management software program EndNote X9 (Clarivate Analytics, Philadelphia, PA) was used to organize and remove duplicate publications retrieved from the four databases.

2.3.2 Study inclusion and exclusion criteria

The inclusion and exclusion criteria for literature retrieval are detailed in Table 2.1. Studies that reported the prevalence of antibodies against *Brucella* spp. in dairy cows in China with explicit diagnostic tests were included. Reviews and clinical reports that only described the clinical symptoms and treatment were excluded because they were not relevant to the scope of this review. An initial screening for inclusion was made according to the titles and abstracts, and publications that reported on other countries, hosts, and diseases were removed immediately.

The full-text manuscripts were then read before the final decision was made to include or exclude them in this review according to the criteria in Table 2.1. Details of the specific reasons for exclusion during the two-step screening process can be seen in Figure 2.1. YW initially conducted the literature selection and data extraction and discussed with other authors to reach a consensus if there were any uncertainties. However, given the language barriers for some authors regarding literature written in Chinese, the main work for retrieved Chinese literature was completed by YW.

Table 2. 1 Inclusion and exclusion criteria used for literature screening in this study

Category	**Inclusion**	**Exclusion**
Study place	China	Other countries
Study population	dairy cows or humans	Other animal hosts
Study time and province	Explicit time and province	Vague time and place
Disease and tests	Investigate brucellosis using explicit tests	Investigate other diseases or vague tests
Study type	Original observational studies	Review and clinical reports
Availability	Full text available	Full text unavailable

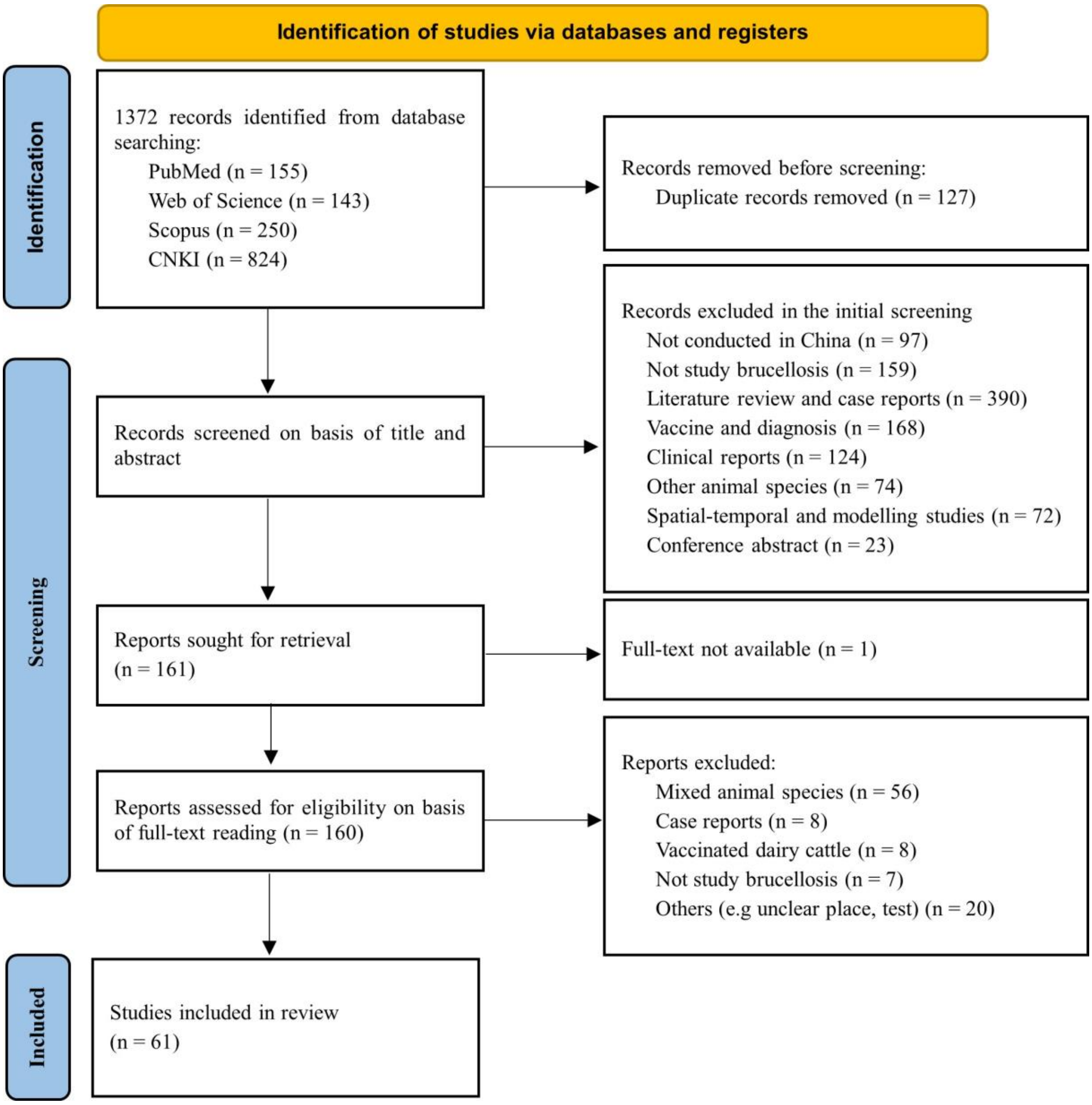

Figure 2. 1 PRISMA flow diagram of the studies identified, screened, assessed, and included in a scoping review of brucellosis in dairy cattle and humans in China.

2.3.3 Data extraction

A template was created to record information about the methodology and results of each publication. The data set documented general study characteristics, including author, publication year, investigation time, province, diagnostic tests used, criteria for positivity, number of units and group (i.e., animals and farms), animal-level and farm-level prevalence, and study type. Sampling times, province, species, biovars of *Brucella* spp., and identification methods were recorded for studies that reported molecular characterization. Only confirmed isolated *Brucella* spp. were counted using relevant species and biovar identification methods. Sample size, study population, study type, and statistical methods were documented for risk factor analysis studies. Data on the monthly number of notified human cases and annual incidence rates from January 2004 to December 2022 were retrieved from the Data Center of China Public Health Science (https://www.phsciencedata.cn/Share/en/index.jsp). Human brucellosis diagnosis and notification protocols have been described elsewhere (Lai et al., 2017; Wang et al., 2021b). The primary criteria for defining a case in the human notification system require meeting at least one of the following conditions:(1) exposure to livestock or contaminated food; (2) pathognomonic clinical signs and symptoms; (3) positive serological tests or bacterial culture. There is no information on the specific *Brucella* species that people are infected with in the human notification system. Province-specific population data were downloaded from the National Bureau of Statistics (https://www.stats.gov.cn/sj/ndsj/) (National Bureau of Statistics of China, 2023).

2.4 Results

2.4.1 Characteristics of included studies

The initial literature search identified 1372 records from four databases, of which 127 were removed for duplication. After screening the titles and abstracts, 1084 of them were rejected for one or more reasons, including mixing species, ambiguous study province or time, vague diagnostic tests, and others, as detailed in Figure 2.1. Literature reviews and case reports

(390/1084) accounted for about a third of the reasons for exclusion. Full-text assessment of the articles was made on 160, and 61 studies were finally enrolled for data extraction (Figure 2.1). Articles written in either Chinese or English were retrieved, of which forty-four (44/61) were in Chinese.

2.4.2 Brucellosis prevalence estimation studies in dairy cattle

Thirty-two reports investigated the prevalence of antibodies against *Brucella* spp. in dairy cattle. None of the serological tests used in China can differentiate *Brucella* species. The main characteristics of these studies are summarized in Table 2.2. These retrieved studies were conducted in 17 of 32 provinces or autonomous regions in China (Figure 2.2). The cross-sectional design was employed in 72% of the prevalence estimation studies, followed by cohort studies. A combined Rose Bengal Test (RBT) and Serum Agglutination Test (SAT) interpreted in series was used in 84% of the studies (27/32) to judge *Brucella* spp. status at the animal level. Fifteen of the 32 studies investigated mixed-type dairy farms (comprising both large-scale and smallholder), but thirty percent of studies did not report herd size. Sampling frame and method were unavailable in 47% of the studies (15/32), and census (6/32) and stratified random sampling (6/32) were the usual methods in the remaining studies. The age of sampled animals was not reported in two-thirds of the studies (21/32), and other studies sampled groups with varying minimum ages, ranging from 3 months to more than 2 years of age. The median of all reported animal-level prevalences was 2.1% (range: 0.0% - 13.5%), while at the farm level its median prevalence was 10.2% (range: 0.0% – 100%). Only half of the studies (16/32) reported farm-level prevalence of brucellosis.

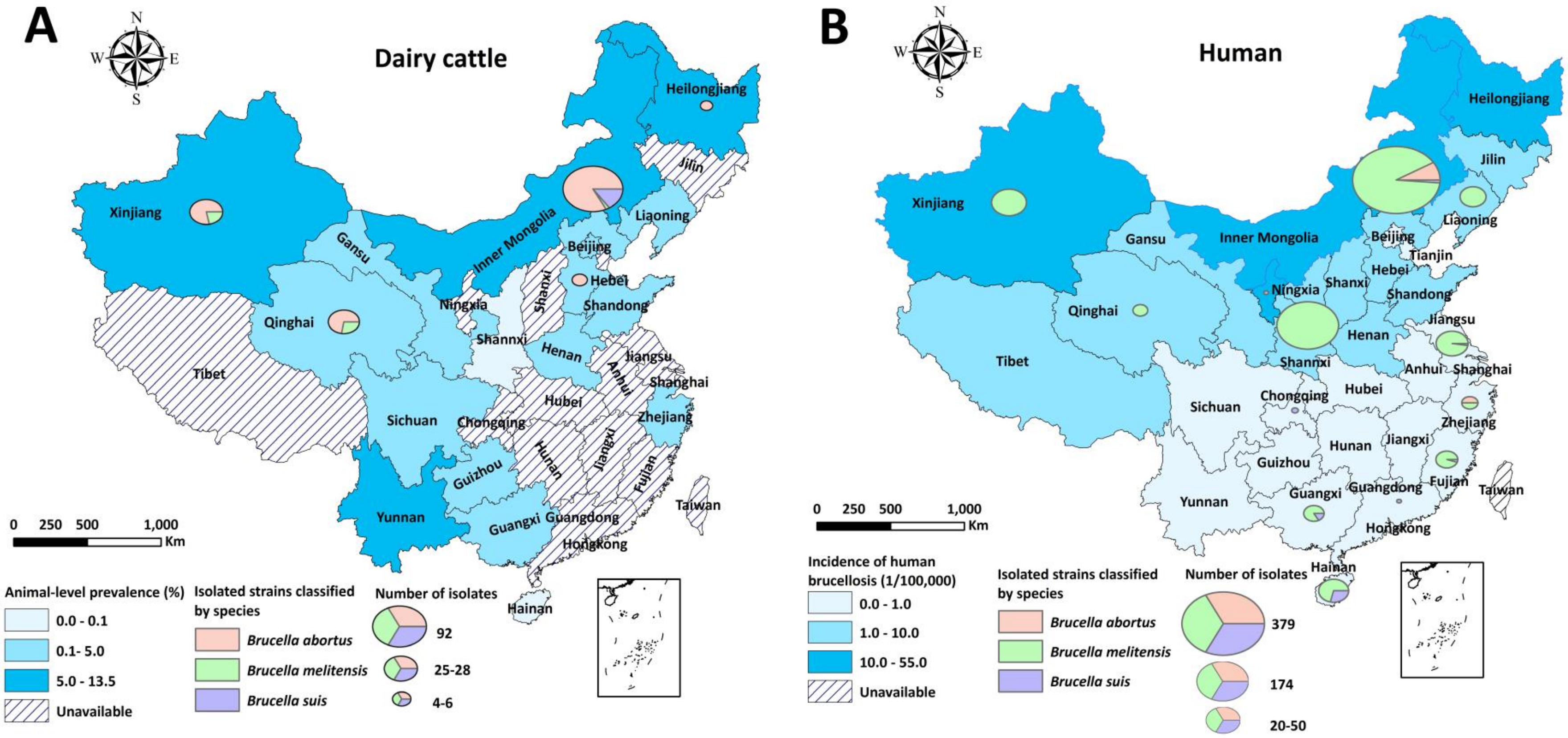

Figure 2. 2 Geographical distribution of reported animal-level prevalence of brucellosis in dairy cattle (Panel A) and incidence of human brucellosis (Panel B) with respective count of *Brucella* spp. isolation and species identification, in provinces in China from 61 studies published between 2004 and 2022.

Table 2. 2 Characteristics of retrieved studies that investigated the seroprevalence of brucellosis in Chinese dairy cows, including study design, geographical location, diagnostic tests, population selection and age, and sample size.

Study period	Study type	Province	Sample type	Diagnostic tests	Herd size[a]	Population selection[b]	Age group	Sample size[c]	Animal-level prevalence (%)	Herd-level prevalence (%)	References
2000-2009	Cohort	Beijing	Blood	RBT + SAT	Mixed	Census	Census	173024	0.1	-	Huang (2012)
2003-2013	Cohort	Guangxi	Blood	RBT + SAT	Not stated	Not stated	Not stated	1039	2.7	-	Jiang et al. (2014)
2004-2010	Cohort	Zhejiang	Blood	RBT + SAT	Mixed	Census	Census	110220	1.5	-	Yu et al. (2011)
2007	Cross-sectional	Zhejiang	Blood	RBT + SAT	Not stated	Not stated	Not stated	1071	2.4	-	Yang et al. (2009)
2007-2011	Cohort	Qinghai	Blood	RBT + SAT	Not stated	Not stated	Not stated	138350	0.3	-	Fu et al. (2012)
2009-2011	Cross-sectional	15 provinces	Milk	PCR	Large-scale	Stratified randomised	> 2 years	5211	1.1	-	Ning et al. (2013)
2009-2011	Cross-sectional	Inner Mongolia	Blood	RBT + SAT	Mixed	Not stated	Not stated	5875 (196)	6.2	26.5	Li (2013)
2009-2013	Cohort	Gansu	Blood	RBT + SAT	Not stated	Not stated	Not stated	10742	2.0	-	Lu et al. (2014)
2012	Cross-sectional	Shandong	Blood	RBT + SAT	Mixed	Census	> 3 months	30119 (1803)	0.4	2.6	Ma et al. (2014)
2012	Cross-sectional	Shandong	Blood	RBT + SAT	Not stated	Not stated	Not stated	333	1.5	-	Meng et al. (2013)
2012	Cross-sectional	Qinghai	Blood	RBT + SAT	Not stated	Stratified randomized	Not stated	18282	0.2	-	Qi et al. (2013)
2012-2013	Cross-sectional	Shandong	Blood	RBT + SAT	Mixed	Simple randomized	Not stated	485 (13)	4.9	15.4	Hao et al. (2014)

Study period	Study type	Province	Sample type	Diagnostic tests	Herd size[a]	Population selection[b]	Age group	Sample size[c]	Animal-level prevalence (%)	Herd-level prevalence (%)	References
2012-2013	Cross-sectional	Heilongjiang	Blood	RBT + SAT	Mixed	Not stated	Not stated	1590	1.1	-	Zhang et al. (2016)
2013	Cross-sectional	Liaoning	Blood	RBT + SAT	Mixed	Census	Census	37888 (187)	0.2	10.2	Cui et al. (2014)
2013	Cross-sectional	Hebei	Blood	RBT + SAT	Large-scale	Not stated	> 6 months	4279 (109)	0.1	5.5	Liu et al. (2016)
2013-2014	Cross-sectional	Heilongjiang	Blood	I-ELISA + C-ELISA	Large-scale	Not stated	Not stated	4131 (22)	12.3	77.3	Liu (2019)
2013-2017	Cohort	Sichuan	Blood	RBT + SAT	Not stated	Stratified randomized	> 12 month	5598	0.2	-	Ran (2018)
2013-2017	Cohort	Gansu	Blood	RBT + SAT	Not stated	Not stated	> 8 months	1431 (160)	4.5	7.5	Wu et al. (2018)
2014	Cross-sectional	Xinjiang	Blood	RBT + SAT	Large-scale	Not stated	> 6 months	987 (3)	6.5	100.0	Huang (2019)
2015	Cross-sectional	Henan	Blood	RBT	Mixed	Census	Census	218 (5)	7.8	100.0	Wan (2017)
2015	Cross-sectional	Yunan	Blood	RBT + SAT	Large-scale	Not stated	Not stated	223	13.5	-	Tao et al. (2016)
2015-2017	Cohort	Sichuan	Blood	RBT + SAT	Mixed	Census	Census	159071 (1157)	0.7	19.5	Chen et al. (2019)
2015-2017	Cross-sectional	Guizhou	Blood	RBT + SAT	Mixed	Simple randomized	Not stated	25910 (148)	0.1	4.1	Li et al. (2019)
2017-2018	Cross-sectional	Xinjiang	Blood	RBT + C-ELISA	Smallholder	Stratified randomized	> 2 years	1406	6.8	-	Zhang et al. (2020)
2017-2019	Cross-sectional	Inner Mongolia	Blood	RBT + SAT	Mixed	Stratified	Not stated	1758	2.7	-	Wu (2021)

Study period	Study type	Province	Sample type	Diagnostic tests	Herd size[a]	Population selection[b]	Age group	Sample size[c]	Animal-level prevalence (%)	Herd-level prevalence (%)	References
						randomized					
2018	Cross-sectional	Xinjiang	Blood	RBT + SAT	Mixed	Simple randomized	Not stated	1203	4.7	-	Qi (2019)
2018	Cross-sectional	Henan	Blood	RBT + SAT	Mixed	Simple randomized	Not stated	25088 (581)	0.8	7.2	Zhao et al. (2020)
2018	Cross-sectional	Shannxi	Blood	RBT + SAT	Not stated	Stratified randomized	Not stated	92 (3)	0.0	0.0	Zhu et al. (2019)
2019	Cross-sectional	Henan	Blood	RBT + SAT	Mixed	Census	Census	12755 (68)	2.2	33.8	Liu et al. (2020a)
2020	Cross-sectional	Hainan	Blood	RBT + SAT	Large-scale	Simple randomized	Census	1690 (2)	0.0	0.0	Ren (2021)
2021	Cross-sectional	Shandong	Blood	RBT + SAT	Mixed	Not stated	Not stated	49080 (1079)	0.5	2.9	Geng (2022)
2021	Cross-sectional	Xinjiang	Blood	RBT + SAT	Not stated	Not stated	Not stated	7102	0.6	-	Wu (2022)

[a]: herd size was categorized as large-scale (number of animals, N≥100), smallholder (N<100), mixed, and not stated; [b]: census suggested all dairy herds and cattle were included in the sampling; [c]: Number of animals tested (Number of farms tested); -: not available; RBT: Rose Bengal Test; SAT: Serum Agglutination Test; C-ELISA: competitive Enzyme-linked Immunosorbent Assay.

2.4.3 Circulating *Brucella* spp. in dairy cattle and humans

A total of 919 *Brucella* spp. were isolated from dairy cattle and humans in 15 provinces of China during 2004-2022 (Table 2.3, Figure 2.2). Most *Brucella* spp. (83.1% - 764/919) originated from humans and 155 from dairy cattle. *B. melitensis* represented 78.6% of the total isolates from humans and dairy cattle, followed by *B. abortus* (18.8%). As for specific hosts, the predominant *Brucella* species prevalent in dairy cows was *B. abortus* (81.9%), while in humans it was *B. melitensis* (92.5%). Further biotyping revealed that *B. abortus* biovar 3 (85.8%) was the primary biotype in dairy cows, whereas *B. melitensis* biovar 3 was the dominant biotype in humans. Spatially, forty-eight percent of *Brucella* spp. (441/919) were isolated from Inner Mongolia. *Brucella* isolates from dairy cows were primarily clustered in Inner Mongolia, while those from humans were collected from Inner Mongolia, Shannxi, and Liaoning (Figure 2.2). Notably, one study isolated 174 *Brucella* spp. in Shannxi Province (An et al., 2022), which accounted for 18.9% of the total (174/919). The year 2008 was chosen as the cut-off for the temporal comparison of isolates, as nearly half of the isolates from dairy cattle were collected before this time. *Brucella* spp. was more often isolated after 2008 than before 2008 (745 vs 174, Figure 2.3), aligning with the number of studies conducted during each period (16 studies vs 6 studies). The proportion of brucellosis attributable to *B. melitensis* in dairy cattle increased markedly from 2.9% before 2008 to 14.9% in 2008-2020 (Figure 2.3 Panel A). In humans, *B. melitensis* accounted for 92.5% of all *Brucella* isolates, followed by *B. abortus* (4.2%). Before 2008, *B. abortus* and *B. melitensis* were the main etiologies of human brucellosis, and *B. melitensis* biovar 3 stood out after 2008 (Figure 2.3 Panel B).

Table 2. 3 Characteristics of 919 *Brucella* spp. isolated from cattle and humans in China

Variables	Host		
	Human (%)	Dairy cattle (%)	Total (%)
Total	764	155	919
Species [a]			
B. abortus	32 (4.2)	127 (81.9)	159 (17.3)
B. melitensis	707 (92.5)	15 (9.7)	722 (78.6)
B. suis	25 (3.3)	13 (8.4)	38 (4.1)
B. abortus biovars [b]			
Biovar 1	5 (15.6)	0 (0.0)	5 (3.1)
Biovar 3	25 (78.1)	109 (85.8)	134 (84.3)
Biovar 6	2 (6.3)	0 (0.0)	2 (1.3)
Unidentified	0 (0.0)	18 (14.2)	18 (11.3)
B. melitensis biovars [b]			
Biovar 1	118 (17.5)	2 (13.3)	120 (16.6)
Biovar 2	9 (1.3)	1 (6.7)	10 (1.4)
Biovar 3	499 (69.2)	5 (33.3)	504 (69.8)
Variant	23 (3.4)	7 (46.7)	30 (4.2)
Unidentified	58 (8.6)	0 (0.0)	58 (8.0)
B. suis [b]			
Biovar 1	7 (28.0)	13 (100.0)	20 (52.6)
Biovar 3	17 (68.0)	0 (0.0)	17 (44.7)
Unidentified	1 (4.0)	0 (0.0)	1 (2.6)
Region [a]			
Inner Mongolia	349 (45.7)	92 (59.4)	441 (48.0)
Shannxi	174 (22.8)	0 (0.0)	174 (18.9)
Other provinces	241 (31.5)	63 (40.6)	304 (33.1)

[a]: the percentage of specific *Brucella* in each host; [b]: the percentage of specific biovar in each specific *Brucella* species within each host, for example, *B. abortus* biovar 3 in humans, 25/32=78.1%.

2.4.4 Risk factors for *Brucella* spp. in dairy cattle

A summary table presents the degrees of strength in dairy cattle in China (Table 2.4), and a directed acyclic graph was constructed to show the potential causal inference for risk factors associated with brucellosis positivity (Figure 2.4). All studies were conducted at the herd level using multivariate logistic regression analysis. The risk of brucellosis in Chinese dairy herds is influenced by management practices, biosecurity practices, and herd characteristics (Cui et al., 2014; Liu et al., 2020a; Ma et al., 2014). Lack of exclusive animal transport vehicles (odds ratio = 3.57), absence of sick animal isolation (OR = 12.89), and failure to quarantine newly introduced animals (OR = 5.50) significantly increase infection risk. In addition, herd characteristics such as open farms (OR = 12.81) and small herds (OR = 4.58) also exhibited high odds of infection compared to closed farms and medium and large herds in China. The presence of dogs within herds and inadequate work hygiene practices (e.g., washing working boots) may facilitate *Brucella* spp. transmission within dairy herds (Wareth et al., 2017). The estimated regional risk of *Brucella* spp. displayed considerable variation, and the seroprevalence of brucellosis in northern provinces was higher than in southern provinces (Figure 2.2). *Brucella* isolates from southern provinces were genetically associated with *Brucella* spp. collected from multiple northern provinces in China (Wang et al., 2020a), suggesting cross-province *Brucella* transmission via animal movements (Zhu et al., 2020).

2.4.5 Public health relevance

Eight studies estimated the seroprevalence of *Brucella* spp. in occupation-associated populations, such as livestock workers or hospitalized patients. In more than half of the studies

(5/8), individuals who tested positive for both RBT and SAT were classified as brucellosis positive. Half of the studies utilized a non-random, convenience sampling method to enroll participants. The median seroprevalence of brucellosis among these investigated people was 3.7% (Range: 1.8 – 16.4) (Table 2.4).

The incidence of notified human brucellosis cases displayed a typical seasonal increase with peaks in June-July (summer) and troughs in December-January (winter) each year (Figure 2.5), followed by a decline during 2015-2018, and another rise through the present (Figure 2.5). The historical peak occurred in 2021, with 73,645 human brucellosis cases notified, equivalent to approximately 5.22 cases per 100,000 person-years (Figure 2.5). Although the average incidence was lower than that in other countries, such as Iraq and Jordan with > 25 cases /100,000 person-years (Dean et al., 2012b), in some regions of China (e.g. Inner Mongolia, 40.9 cases/100,000 person-years), it was comparable to severely affected countries (Wang et al., 2021b). A higher incidence of human brucellosis was found among occupation-related populations, males aged 35-54, and those residing in specific geographical areas (e.g., Inner Mongolia) (Lai et al., 2017; McDermott et al., 2013; Wang et al., 2021b). For occupationally at-risk groups, behaviors such as consuming raw milk, assisting in calving, and handling abortions without appropriate PPE were risk factors for *Brucella* infection (Wang et al., 2021a). In contrast, frequent disinfection of calving sites and proper disposal of aborted calves were identified as protective factors. Additionally, some meteorological and geographic factors, such as moderate altitude, grassland, medium temperatures, and reduced sunshine, were significantly associated with the incidence of human brucellosis (McDermott et al., 2013; Peng et al., 2020a).

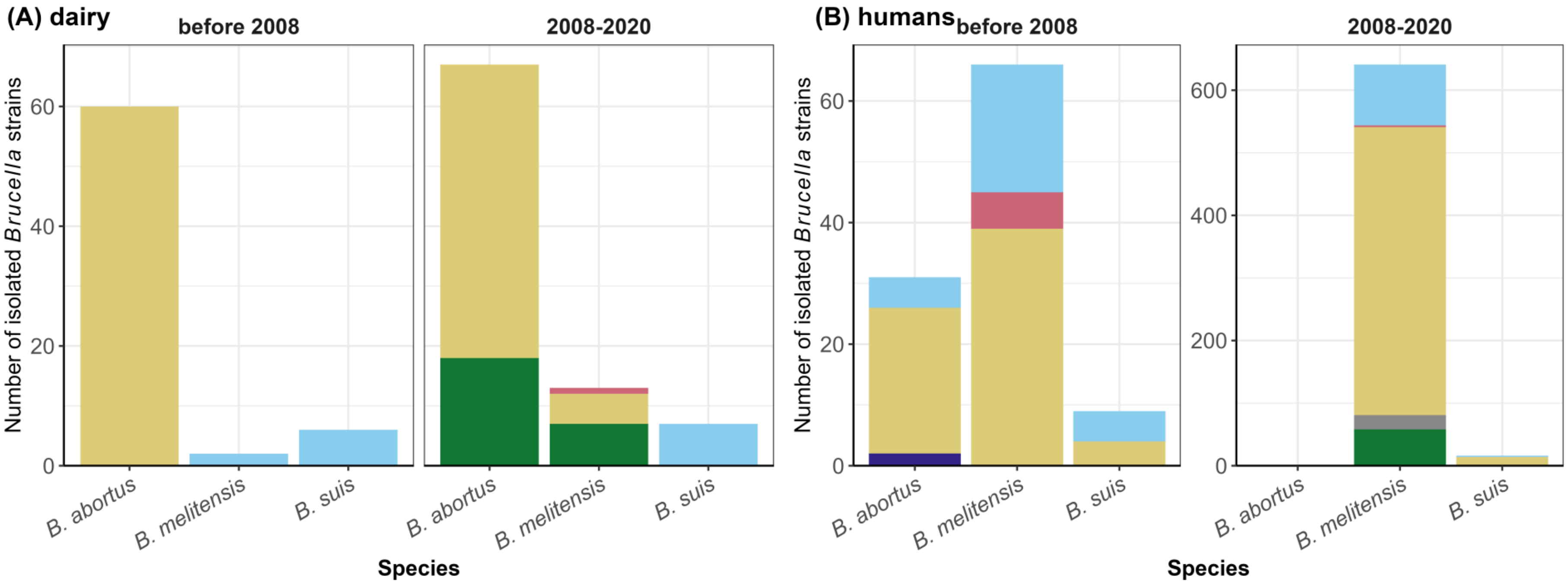

Figure 2. 3 Species and biovars of *Brucella* spp. isolated from dairy cattle (Panel A) and humans (Panel B) published between 2004 and 2022, bv represents biovar.

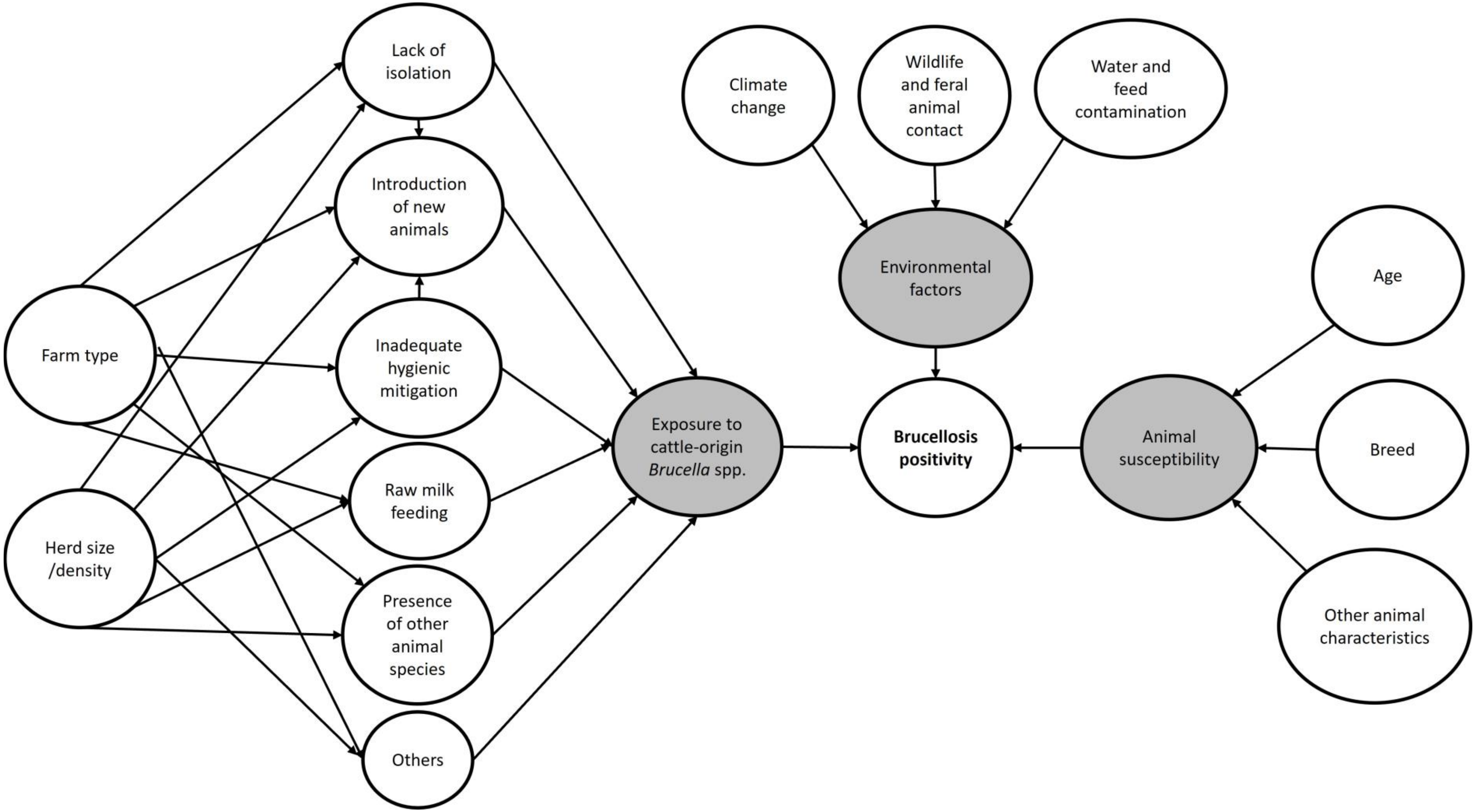

Figure 2. 4 Directed acyclic graph shows the potential causal pathways for brucellosis positivity in dairy herds. The solid thick black lines denote the "direct pathway", and grey nodes denote unmeasured variables.

Table 2. 4 A summary of risk factors associated with brucellosis in dairy herds in China from retrieved reports.

Variable	Study unit	Odds ratio	95% CI	*p*-value	Reference
Exclusive animal transport vehicles: No vs Yes	Herd-level	3.57	1.04 – 12.5	0.04	Cui et al. (2014)
Wearing working clothes and boots: No vs Yes	Herd-level	5.00	0.97 – 25.00	0.06	Cui et al. (2014)
Observation of dogs within herd: Yes vs No	Herd-level	12.39	0.97 – 157.56	0.05	Cui et al. (2014)
Isolation of sick animals: No vs Yes	Herd-level	12.89	1.67 – 99.33	0.014	Liu et al. (2020a)
Farm type: Open vs Closed	Herd-level	12.81	2.11 – 77.83	0.005	Liu et al. (2020a)
Quarantine for introduced animals: No vs Yes	Herd-level	5.50	0.73 – 41.40	0.008	Liu et al. (2020a)
Herd size: 1-10 vs 11-500	Herd-level	4.58	1.87 – 11.23	0.001	Ma et al. (2014)
Farming type: Dairy farming village vs intensive dairy farm	Herd-level	2.21	1.05 – 4.63	0.036	Ma et al. (2014)

Note: A dairy farming village is a special farming community that contains several independent dariy farming units and shares milking and feeding facilities; an intensive dairy farm is an independent dairy herd under intensive management.

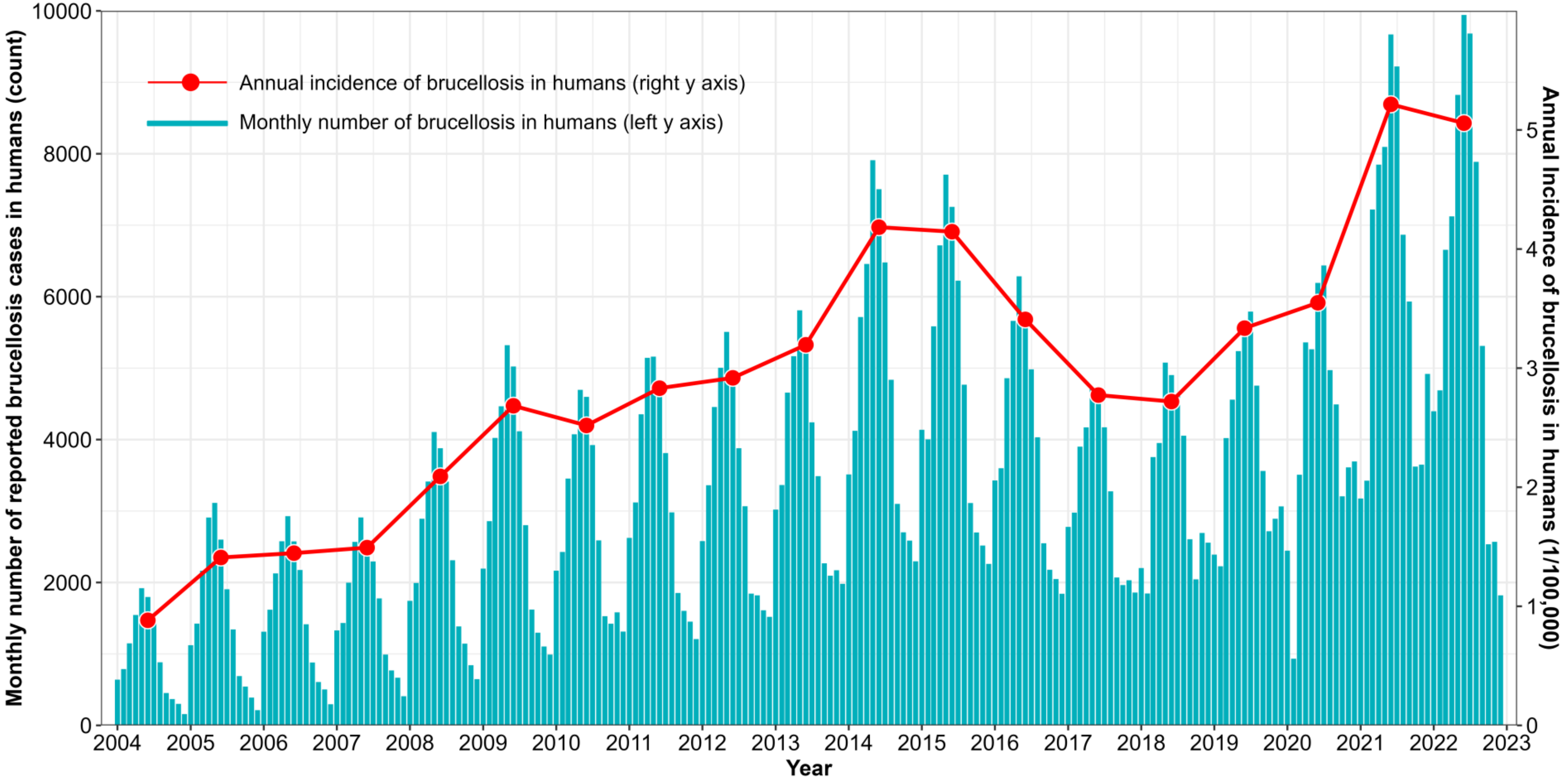

Figure 2. 5 Monthly number of human brucellosis notifications in China between January 2004 and December 2022 (cyan bar: monthly number of cases by the left y-axis, red line: annual incidence of brucellosis in humans by the right y-axis, 1/100,000 people).

Table 2. 5 Study characteristics of seroprevalence of *Brucella* infection in Chinese humans

Study period	Study type	Province	Tests	Source population	Population selection method	Sample size	Prevalence (%)	References
2004-2010	Cohort	Zhejiang	RBT+SAT	Occupation-associated	Convenient sampling	10430	2.0	Yu et al. (2011)
2005	Cross-sectional	Fujian	SAT	Occupation-associated	Convenient sampling	1321	3.2	Zhuo et al. (2007)
2008-2020	Cohort	Shannxi	RBT+SAT	Occupation-associated	Convenient sampling	179907	4.3	An et al. (2022)
2010-2012	Cohort	Sichuan	RBT+SAT	Occupation-associated	Convenient sampling	450	4.4	Zhou et al. (2014)
2010-2014	Cohort	Inner Mongolia	SAT	Occupation-associated	Stratified randomized sampling	838956	3.6	Ning et al. (2018)
2012-2016	Cohort	Inner Mongolia	RBT+SAT	Suspected populations	Convenient sampling	1102304	3.8	Liu et al. (2020c)
2013	Cross-sectional	Inner Mongolia	RBT+SAT	Occupation-associated	Census sampling	13098	1.8	Kang (2014)
2014-2021	Cohort	Shannxi	RBT+SAT	Occupation-associated	Convenient sampling	4263	1.4	Zhao et al. (2022)
2016	Cross-sectional	Jiangsu	RBT+SAT	Occupation-associated	Convenient sampling	895	16.4	Xiao et al. (2016)
2016-2020	Cohort	Fujian	SAT	Occupation-associated	Not available	4934	1.7	Han et al. (2022)
2019-2020	Cross-sectional	Shanxi and Xinjiang	SAT	Occupation-associated	Simple randomized sampling	2384	2.6	Lin et al. (2021)

RBT: Rose Bengal Test; SAT: Serum Agglutination Test.

2.5 Discussion

This scoping review provides a comprehensive overview of studies investigating the prevalence of antibodies against *Brucella* spp. and the distribution of *Brucella* species and biovars in dairy cattle and humans from literature published between 2004 and 2022. Of the 1372 studies retrieved, only 61 met the inclusion criteria in this review, with the majority being excluded due to unrelated research topics, heterogeneous quality of study reporting, and inexplicit or missing information (Figure 2.1). Many studies were published in local journals and non-English languages, limiting accessibility to international researchers. By combining these fragmented data sources, this review strengthens our understanding of *Brucella* infection in dairy cattle and humans and highlights critical gaps in research and disease control strategies in China.

Despite the recognition of *Brucella* spp. as a significant zoonotic pathogen, research and control efforts in China have predominantly focused on *B. melitensis* in small ruminants (Li et al., 2020b; Zhu et al., 2020), while *B. abortus* in dairy cattle has received less attention and effort than warranted. Justifying *B. abortus* becomes essential within a One Health framework of brucellosis control in China, especially considering the prevailing gaps in understanding the relative importance of different *Brucella* species in cows and humans within China. Given the rapid expansion of China's dairy industry and booming milk demand, addressing *Brucella* spp. is critical for both dairy cattle and human health in China. This review underscores the need for an integrated approach to brucellosis surveillance and control, particularly in bridging the gap between animal and human health sectors.

Although the One Health approach has been widely advocated for zoonotic disease management globally (One Health High-Level Expert et al., 2022; Zinsstag et al., 2011), none of the 61 studies explicitly incorporated this framework into their studies, suggesting a major disconnect between research on animal and human brucellosis in China. While studies have emphasized the need for a One Health approach (Lai et al., 2017; Wang et al., 2021b), there is little evidence of coordinated efforts across multisectors to implement such strategies in China. Pepin et al. (2024) identified essential challenges, solutions, and priorities for coordinating a One Health program through government action, and we recommend adjusting these recommendations to accommodate unique local socioeconomics, husbandry management, and *Brucella* epidemiology.

Brucellosis has been recognized as a priority disease by the Ministry of Agriculture and Rural Affairs (MARA) of China, given the notable increase in notifications among humans and reported prevalence in livestock (Ministry of Agriculture and Rural Affairs, 2022). Since 2004, the notified brucellosis cases in humans and animals exhibited key characteristics: (1) a substantial increase in incidence and prevalence (Figure 2.5); (2) seasonal fluctuations with high incidence in summer and low incidence in winter (Figure 2.5); (3) spatial expansion from northern to southern provinces (Lai et al., 2017; McDermott et al., 2013; Wang et al., 2021b). These trends are likely driven by multiple factors, including (1) increased stocking density and associated higher contact frequency between animals, accelerating the within-herd transmission of infectious diseases (Deqiu et al., 2002; McDermott et al., 2013); (2) thriving inter- and intra-provincial live animal trade and movement, which likely facilitated disease transmission between herds (Wang et al., 2021b); (3) low vaccination coverage, failing to establish sufficient herd immunity to resist *Brucella* (re-)invasion (Chen et al., 2021); (4) inadequate financial resources, making test-and-slaughter strategies impractical and unaffordable (Chen et al., 2021); (5) vulnerable on-farm biosecurity measures, struggling to prevent pathogen (re-)invasion (Robertson, 2020); (6) limited farmer awareness about the public health and economic significance of *Brucella*, compromising the adoption of disease control measures (Zhang et al., 2019). However, these factors may not fully explain the observed fluctuations in brucellosis notifications in humans between 2015 and 2022. Financial limitations and competing priorities, such as the current COVID-19 pandemic, may exacerbate the challenges associated with controlling and preventing brucellosis in China. The limited financial resources available to implement effective control measures, coupled with a focus on the COVID-19 response, may further hinder the prevention and control of brucellosis after January 2020.

The median reported animal-level seroprevalence of brucellosis in dairy cattle in China was 1.5%, similar to previous estimates (Ran et al., 2019). However, the median reported farm-level prevalence reached 10.2%, indicating that a considerable proportion of dairy populations were affected by *Brucella*. Although these prevalences were lower than those reported in endemic countries such as India (animal-level: 15.1%, herd-level: 32.9%) (Holt et al., 2021) and Ethiopia (2.6% and 16.3%) (Sibhat et al., 2022), or other countries in the early eradication stage like New Zealand (15% and 59%) (Adlam, 1978) and Australia (Lehane, 1996), China still confronts a significant challenge in controlling brucellosis because of its sizable dairy population and complex conditions.

Only half of the prevalence estimation studies reported farm-level prevalence of *Brucella* infection in the dairy sector. The lack of farm-level prevalence data weakened epidemiological understanding of disease status at the farm level and potentially impaired policy-making for control measures, as interventions were typically designed and implemented at the farm level (Zhang et al., 2018). Possible reasons for this underreporting include a lack of emphasis on farm-level prevalence in study designs and concerns that reporting high prevalence may raise public concern and economic repercussions for the dairy industry (Chen et al., 2021). To address this, transparent farm-level prevalence reporting with detailed information should be strengthened for a robust understanding of the disease status in the population.

Prevalence estimates are often influenced by various factors such as sampling methods, target populations, testing assays, and case definitions. Small herd size has been reported to be a risk factor for *Brucella* positivity at the farm level in some studies (Cui et al., 2014; Liu et al., 2020a) but not in other international studies showing increased risk with large herd size (de Alencar Mota et al., 2016). Differences in livestock management practices and levels of farm biosecurity may explain this discrepancy (Chen et al., 2021). The age of the animals sampled is essential since older animals are expected to have a longer exposure time, making them more likely to be positive for *Brucella* spp. (McDermott et al., 2013; Wang et al., 2021a; Zeng et al., 2017; Zhang et al., 2020). Unfortunately, many prevalence estimate studies have not mentioned the age of animals, which may introduce biases that cannot be accounted for. Additionally, provinces with a high disease prevalence, like Inner Mongolia and Shannxi province, were overrepresented in research reports, while the lack of brucellosis data in these unreported regions highlights the need for greater efforts in reporting true *Brucella* spp. status.

Using RBT and SAT in series is commonly used to judge the serological status of *Brucella* exposure in dairy cattle in China (Table 2.2) (Lai et al., 2017). However, this testing strategy prioritizes overall specificity over sensitivity, leading to underestimation of true prevalence due to false negatives (Ducrotoy et al., 2018; Wang et al., 2021b). False-negative results may allow infected cattle to persist within farms, maintaining the endemicity of *Brucella* spp. and facilitating transmission to other susceptible animals or farms (Bercovich, 1998; Capparelli et al., 2009). Alternative testing strategies should be adopted based on specific epidemiological goals. A serial testing strategy is economically justified to avoid false positives at the animal level, particularly for test-and-slaughter policies. Conversely, prioritizing the identification of positive farms may favor parallel testing to enhance the possibility of detecting positive farms at the early stage of a control program. Furthermore, incorporating polymerase chain reaction (PCR) or bacterial culture techniques when serology suggests a positive result can help identify species and biovar of *Brucella* (Khurana et al., 2021).

This review revealed that *B. abortus*, specifically *B. abortus* biovar 3, was the most commonly identified *Brucella* spp. in Chinese dairy farms. However, the research on *Brucella* isolation and identification for Chinese dairy populations was still limited compared to humans as shown in Figure 2.2. The isolation of *Brucella* in dairy cattle has only been reported in five provinces, possibly due to the lack of biosafety level 3 laboratories and trained professionals required to work with this organism in other provinces. It is crucial to update our knowledge of the species and biovars of currently circulating *Brucella* spp. in unreported provinces (Figure 2.2A). Henan Province is one of the provinces that has not reported the isolation or identification of *Brucella*, so we suggest that work in this province needs to be strengthened in Henan to address this knowledge gap. Molecular epidemiology is essential for enhancing our understanding of brucellosis transmission and contact patterns in endemic regions that maintain molecular databanks of circulating *Brucella* spp. (Liu et al., 2020c). Researchers can infer transmission timing and direction by examining the molecular features and evolutionary relationships of these isolates from different farms or regions (Li et al., 2020b; Ma et al., 2020; Wang et al., 2020a). Investigating these epidemiological links will allow for more informed inferences about the most critical transmission routes in prevalent areas, enabling evidence-based biosecurity recommendations tailored to individual farms to help mitigate the risk of disease introduction.

B. melitensis has been identified as the predominant *Brucella* spp. that causes human brucellosis in China (Figure 2.2B & Figure 2.3B), aligning with previous research findings (Zhu et al., 2020). The higher virulence and infectivity of *B. melitensis* and more potential contacts with *B. melitensis* hosts (e.g., sheep and goats) may account for this dominance (Pappas et al., 2005; WOAH, 2023b). The seasonal grazing and lambing patterns of small ruminants are likely to be associated with the temporal changes in the incidence of notifications in humans, but exploring their association is beyond the scope of this review. For the general public, foodborne infection is the most common route, with infections from the consumption of raw milk frequently reported (Piao et al., 2020). Occasional *Brucella* vaccine leaks have also been reported in China (Baoshan et al., 2021; Pappas, 2022), highlighting the need to improve laboratory biosafety management. Among eleven provinces, 764 *Brucella* spp. were isolated from humans, with most of these isolates clustered in the Inner Mongolia and Shannxi Provinces (Figure 2.2B).

Consequently, caution should be exercised in generalizing the current findings to other provinces, as the heterogeneous distribution of *Brucella* isolates across provinces may impact the broader applicability of the results (Figure 2.2).

This review identified the introduction of new animals, open farming, and poor hygienic practices as potential risks for the between-farms transmission of *Brucella* spp and other infectious diseases (Cui et al., 2014; Liu et al., 2020a; Wang et al., 2020c). Understanding these factors is essential when tailoring a risk-based control scheme in China. Even if a farm has achieved brucellosis-free status, the risk of transmission between farms remains if farms are still connected via other routes (e.g., animal movements) (Cui et al., 2014). The imbalance of meat and milk consumption, cattle population, and animal market price differences across Chinese provinces facilitate inter- and intra-provincial movement or trade of live animals and animal products. Although studies have investigated animal movement and trade patterns between Chinese pig farms and markets regarding other infectious diseases (Li et al., 2020a; Shi et al., 2021), the impact of cattle movements on the transmission of *Brucella* spp. remains unexplored in China. Assessing the effectiveness of the current measures to prevent *Brucella* spp. spread through live cattle movements should be a priority to develop more targeted and effective control measures (Li et al., 2020a; Marquetoux et al., 2016).

In March 2022, the MARA of China launched a five-year action plan for the prevention and control of animal brucellosis (2022-2026) (Ministry of Agriculture and Rural Affairs, 2022). The plan encompasses five key principles for controlling brucellosis in dairy herds: (1) identifying and eliminating *Brucella*-infected animals with appropriate compensation to disrupt the within-herd transmission cycle, (2) mandating vaccination for brucellosis-positive dairy farms to reduce disease prevalence, (3) implementing pre-movement testing of animals and preventing live animal movements from vaccinated areas (high-risk) to non-vaccinated areas (low risk), (4) maintaining continuous surveillance to maintain brucellosis-free status in farms that have achieved freedom, and (5) disseminating disease knowledge to improve farmers' awareness towards the disease. These measures align with the findings of this review and are anticipated to mitigate the impact of *Brucella* spp. on dairy farms and public health. Available links in *Brucella* spp. between human and animal cases are still limited, making it difficult to track and eliminate the source of infection in livestock. Only joint, scientific, evidence-based contributions should be conducive to effective and resonant control strategies.

Despite the countermeasures adopted by the Chinese government over the past decades, brucellosis remains prevalent in China, posing ongoing challenges for public health, veterinary authorities, and farm stakeholders. A successful disease control and prevention program requires collaborative efforts from public health, veterinary departments, and stakeholders. Adequate financial resources are critical, particularly for the test-and-slaughter approach, and appropriate compensation for culled animals serves as a key motivator for stakeholders to report cases and comply with control measures. Furthermore, as mandated by government policy, transparent reporting of vaccination information and effective tracking of vaccinated animals is essential to prevent animal movements from vaccinated areas to non-vaccinated areas. The Chinese government is already promoting the use of electronic tracking systems to replace the original paper documents to record animal movements. More data in the future will allow effective monitoring of animal movements and the successful establishment of epidemiological links between animals and humans. However, the limited number of veterinary professionals and the large population of China constrain the capacity of surveillance and control programs to conduct farm censuses, farm registration, vaccination records, animal movement tracking, data collection, and stakeholder communication. The heterogeneity across regions and the lack of transparent and high-quality reporting further hinder stakeholders' judgment of the priority and importance of the disease. Addressing these challenges requires joint efforts, financial resources, and professional expertise to effectively tackle the increasing prevalence of *Brucella* spp. for dairy cattle where isolation and identification of *Brucella* spp. were much less frequent than in humans in China (Figure 2.3). As new policies are implemented, the self-determination to adopt either vaccination or test-and-culling measures will depend strongly on farm decision-makers' awareness and understanding of brucellosis, and further scientific evidence is required to guide farmers in making evidence-based decisions.

2.6 Conclusions

In conclusion, this scoping review provides a comprehensive summary of the status of *Brucella* spp. for dairy cattle and humans and highlights critical gaps in China's brucellosis control programs, including unadjusted seroprevalence, insufficient reporting of farm-level prevalence, and heterogeneous study reporting and design. While control measures and tools are in place, improvements can be made to enhance the effectiveness of *Brucella* spp. control programs in terms of diagnostic methods, farm biosecurity, and disease impact. Future research could focus on diagnostic evaluation, farm biosecurity practices, disease impact, and financial return of brucellosis control programs to inform more targeted and sustainable interventions.

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Yu Wang
Name and title of main supervisor:	Dr Chris Compton
In which chapter is the manuscript/published work?	Chapter 3

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Yu Wang: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. Compton Chris: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. Emilie Vallée : Methodology, Supervision, Writing – review & editing. Cord Heuer: Methodology, Supervision, Writing – review & editing. Aizhen Guo: Funding acquisition, Investigation, Supervision, Writing – review & editing. Youming Wang: Methodology, Supervision, Writing – review & editing. Zhen Zhang: Investigation, Resources, Supervision. Matthieu Vignes: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – review & editing.

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Doctoral Research Committee
May 2023

Chapter 3 A novel Bayesian Latent Class Model (BLCM) evaluates multiple continuous and binary tests: A case study for *Brucella* spp. in dairy cattle

Yu Wang [a], Emilie Vallée [a], Chris Compton [a], Cord Heuer [a], Aizhen Guo [b], Youming Wang [c], Zhen Zhang [d], Matthieu Vignes [e]

[a] EpiCentre, School of Veterinary Science - Tāwharau Ora, Massey University, Palmerston North, New Zealand

[b] National Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China

[c] China Animal Health and Epidemiology Center, Qingdao, China

[d] Henan Dairy Herd Improvement Center, Zhengzhou, Henan, 450045, China

[e] School of Mathematical and Computational Sciences, Massey University, Palmerston North, New Zealand

Preface

Effective control of bovine brucellosis depends on the identification of infected cattle and herds, which requires an accurate diagnosis of infected animals. However, studies evaluating the diagnostic performance of diagnostic tests remain limited in dairy herds in China, and adjustments for imperfect diagnostic sensitivity and specificity in prevalence estimation are often overlooked. Additionally, when two or more tests based on the same detection principle (e.g., antibody-based) are used in series or parallel, conditional dependencies are widely omitted in adjusting overall diagnostic sensitivity and specificity in China (Wang et al., 2024b), which likely results in diagnostic errors in interpreting test results.

Chapter 2 highlighted that no prevalence studies accounted for diagnostic test accuracy from 2004 to 2022, potentially compromising estimates of true disease burden and geographic distribution. To address this gap, Chapter 3 presents a comprehensive evaluation of four serological tests widely used in the diagnosis of *Brucella* spp. infection in dairy cattle in Henan Province. A Bayesian latent class model was developed, incorporating both fixed and random effects to account for test dependencies. Two binary and two continuous antibody-detection tests were analyzed, with optimal cut-off values determined by maximizing the sum of sensitivity and specificity. The findings provide a more accurate classification of *Brucella* infection status at the individual level, improve disease prevalence estimation, and support broader epidemiological applications. These include demonstrating disease freedom (Pei et al., 2025), and informing financial analyses (Chapter 6), ultimately contributing to more effective brucellosis control strategies.

3.1 Abstract

Bovine brucellosis, primarily caused by *Brucella abortus*, severely affects both animal health and human well-being. Accurate diagnosis is crucial for designing evidence-based control and prevention measures. Lacking a gold standard test makes it challenging to determine optimal cut-off values and evaluate the diagnostic performance of tests. In this study, we developed a novel Bayesian Latent Class Model that integrates both binary and continuous testing outcomes, incorporating additional fixed (parity) and random (farm) effects, to calibrate optimal cut-off values by maximizing Youden Index. We tested 651 serum samples collected from six dairy farms in two regions of Henan Province, China with four serological tests: Rose Bengal Test, Serum Agglutination Test, Fluorescence Polarization Assay (FPA), and Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA). Our analysis revealed that the optimal cut-off values for FPA and C-ELISA were 94.2 mP and 0.403 PI, respectively. Sensitivity estimates for the four tests ranged from 69.7% to 89.9%, while specificity estimates varied between 97.1% and 99.6%. The true prevalences in the two study regions in Henan province were 4.7% and 30.3%. Parity-specific odds ratios for positive serological status ranged from 1.2 to 2.1 for different parity groups compared to primiparous cows. This approach provided a robust framework for validating diagnostic tests for both continuous and discrete tests in the absence of a gold standard test. Our findings can enhance our ability to design targeted disease detection strategies and implement effective control measures for brucellosis in Chinese dairy farms.

Keywords: bovine brucellosis, Bayesian Latent Class Model (BLCM), Cut-off estimation, Receiver Operating Characteristic (ROC), diagnostic performance, serological tests

3.2 Introduction

Bovine brucellosis, primarily caused by *Brucella abortus* (*B. abortus*), is a significant disease of both economic and public health concern globally, especially in the Middle East and parts of Asia, including China (Pappas et al., 2006; Wang et al., 2020b; Zhang et al., 2018). In dairy cattle, brucellosis mainly affects sexually mature cows and manifests through late-trimester abortions, infertility, and reduced milk yield (Neta et al., 2010). Alarming findings from recent studies indicate an upward trajectory in the animal-level prevalence of this disease within Chinese dairy populations (Ran et al., 2019). Such an escalating prevalence potentially poses severe consequences for affected herds and has profound implications for production within the Chinese dairy industry, ultimately affecting public health in China. Transmission of the disease to humans mainly occurs through the consumption of undercooked meats or raw dairy products originating from infected animals (Bercovich, 1998), direct contact with contaminated aborted products, and inhalation of contaminated aerosols (Pappas, 2022). Consequently, implementing comprehensive control measures in livestock has been highlighted as a valuable and economically rewarding strategy, preventing the dissemination of the pathogen to consumers and the public (Zinsstag et al., 2007).

Diagnostic tests are essential tools for decision-makers to confirm or inform the infection status of individual animals or farms, estimate disease prevalence, demonstrate disease freedom, and conduct epidemiological studies. Direct detection of *Brucella* spp. can be achieved through bacterial culture and biochemical characteristics (e.g., CO_2 requirement and H_2S production) or polymerase-chain-reaction (PCR) tests (WOAH, 2023b). While bacterial culture and biochemistry are considered the 'gold standard' for confirming *Brucella* spp. infection, they are influenced by factors including bacterial viability, tissue concentration, environmental contamination, shedding period, and the need for an accredited biosafety level 3 laboratory (Mantur et al., 2007; Rahman et al., 2019; WOAH, 2022b). Furthermore, these methods are also imperfect, expensive, and time-consuming, and hence not suitable for routine disease surveillance (Ducrotoy et al., 2018; WOAH, 2022b). Indirect testing of anti-*Brucella* antibodies in serum and milk samples is preferred in routine diagnosis and surveillance (Ducrotoy et al., 2018; Wang et al., 2020c; WOAH, 2022b). Serological tests are advantageous due to their low cost, rapidity, ease of operation, and high diagnostic sensitivity (*Se*) and specificity (*Sp*). In dairy cattle, detectable levels of IgM antibodies against *Brucella* spp. are typically reached one-week post-infection, while IgG antibodies become detectable by two weeks post-infection and are considered to persist lifelong (Baldi et al., 1996; Bercovich, 1998). Serological tests are particularly valuable in diagnosing animals that display clinical signs, such as abortion, as well as in screening apparently healthy individuals. By employing these tests, it is possible to identify exposed animals and conduct surveillance efficiently and effectively.

Serological tests such as the Rose Bengal test (RBT) and Serum agglutination test (SAT) are commonly used in epidemiological surveys to assess the seroprevalence of *Brucella* in dairy cattle in China (Ran et al., 2019). However, only a few studies have adjusted these estimates to account for the imperfect *Se* and *Sp* of these tests in China (Greiner et al., 2000a; Wang et al., 2024b). Although RBT and SAT are considered accurate, their results are acquired by naked-eye observation and are therefore subject to the judgment and experience of operators, particularly for suspicious samples (Dong et al., 2021; Ducrotoy et al., 2018). Fluorescence polarization assay (FPA) and competitive enzyme-linked immunosorbent assay (C-ELISA) have been developed more recently and can offer the advantage of providing quantitative and automated result readings, making them suitable for large-scale investigations. While FPA and C-ELISA kits are commercially available in China, their thresholds, *Se*, and *Sp* have not been extensively calibrated. Many existing diagnostic evaluation studies have used SAT as reference tests to obtain a relative *Se*, *Sp*, and agreement with other tests; however, these studies often failed to account for the imperfect *Se* and *Sp* of the reference test itself (Dong et al., 2021; Yao et al., 2022). Alternatively, some studies have utilized serum samples from naturally infected animals or clinical challenge trials, as well as disease-free populations, but these settings cannot fully replicate natural infection scenarios (Greiner et al., 2000a, 2000b).

Bayesian latent class models (BLCMs) have emerged as a valuable and recommended approach for estimating the accuracy of diagnostic tests when a 'gold standard' test is unavailable (Cheung et al., 2021; Gardner et al., 2019; WOAH, 2022a). In BLCMs, disease status is treated as unknown or 'latent', and *Se* and *Sp* estimates are derived from the cross-classified test results being evaluated in one or more population(s) (Cheung et al., 2021; Kostoulas et al., 2017). Bayesian analysis allows for the incorporation of prior knowledge or existing information about parameters, facilitating the derivation of posterior distributions of *Se* and *Sp* (Cheung et al., 2021; Kostoulas et al., 2017). Many studies have utilized BLCMs to estimate the diagnostic *Se*, *Sp* of serological tests, and prevalence of *Brucella* spp. exposure in dairy cattle using dichotomous data (Arif et al., 2018; Rahman et al., 2019; Wang et al., 2020c). Moreover, the application of modeling continuous test outcome data in veterinary studies has been well-developed, as it avoids the loss of information resulting from dichotomization and enables the estimation of optimal cut-off values and associated *Se* and *Sp* (Choi et al., 2006; Jones et al., 2009; Yang et al., 2022b). However, there are no studies combining several continuous and discrete tests for joint analysis in a BLCM. Covariates such as lactation stage and age have been extensively identified to be associated with *Brucella* spp. infection (Matope et al., 2011), and Jones et al. (2009) demonstrated that incorporating covariates could contribute to accurate disease diagnosis.

Our study aimed to develop a novel BLCM for modeling dichotomous and continuous test outcome data to estimate: (1) optimal thresholds of FPA and C-ELISA; (2) *Se* and *Sp* of four commonly used serological tests in China – RBT, SAT, FPA, and C-ELISA; (3) the animal-level prevalence of brucellosis in selected dairy farms; (4) odds ratio for age (parity) on serological status of brucellosis. We followed the Standards for Reporting of Diagnostic Accuracy studies that use Bayesian latent class models (STARD-BLCM) guidelines in reporting this study (Kostoulas et al., 2017).

3.3 Materials and Methods

3.3.1 Study design and samples

The study was conducted in the Pingdingshan region (PR) and Zhengzhou region (ZR) of Henan province, China, as shown in Figure 3.1. The geographic coordinates of the study area lie between latitudes 112°14′ and 114°13′E and longitudes 33°07′ and 34°59′N. Dairy cattle were managed intensively in both regions, coupled with crop-based management systems. The Holstein-Friesian breed was exclusively raised in these areas. Previous investigations have reported apparent prevalences of antibodies against *Brucella* spp. at the cow level as 2.2% (95% CI: 2.0, 2.5) in PR and 7.8% (95% CI: 4.6, 12.2) in ZR (Liu et al., 2020a; Wan, 2017).

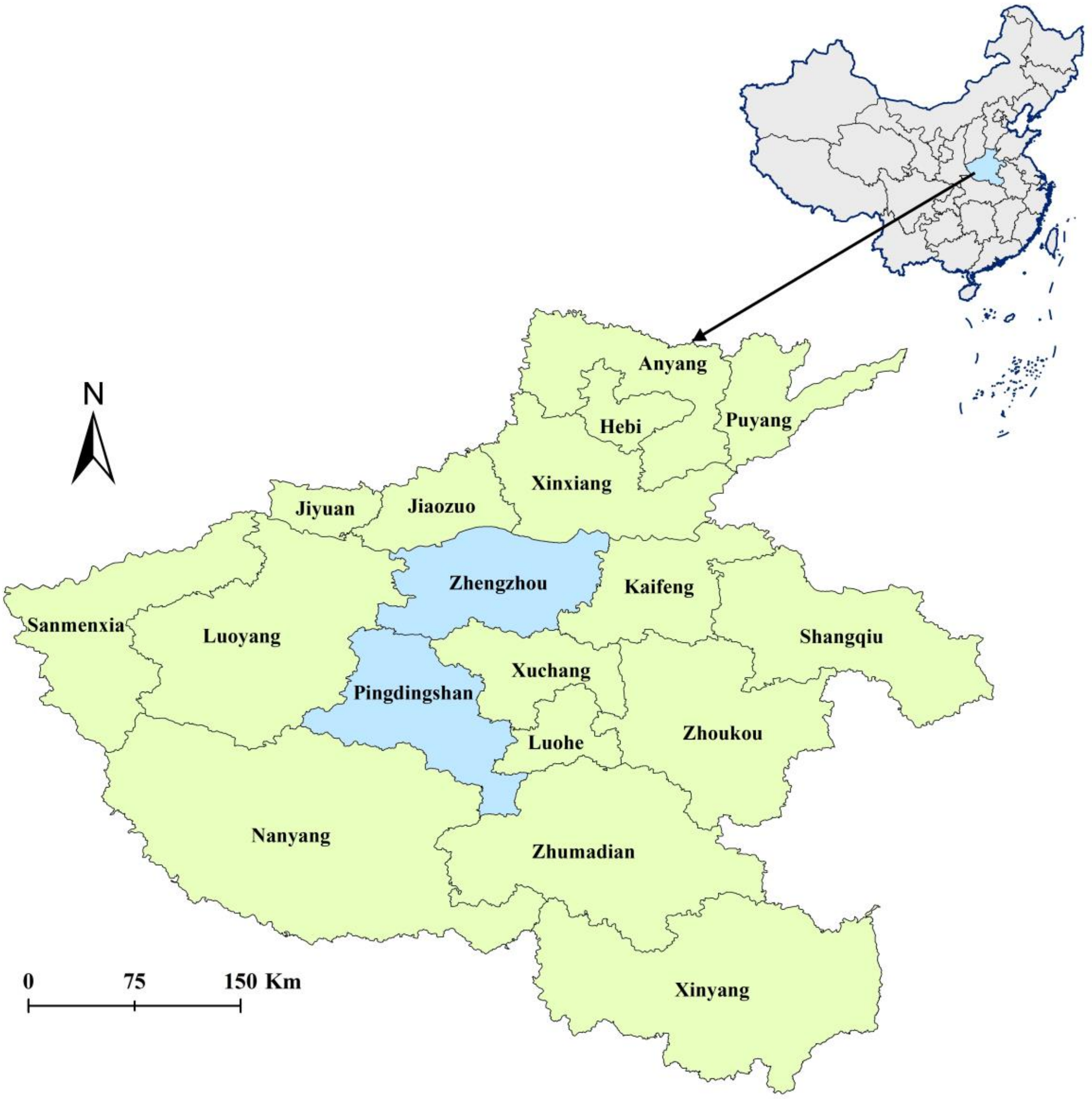

Figure 3. 1 Map of Henan Province, China displaying the areas included in this diagnostic performance evaluation study

A cross-sectional study was undertaken between August 2021 and December 2021 in PR and

ZR regions of Henan Province. Farmers or their veterinarians collected blood samples as part of routine early pregnancy testing, taken 28 days after artificial insemination. These samples, originally intended for pregnancy diagnosis, were repurposed for this study. Twenty-four dairy herds in two regions were initially enrolled in the selection of potential herds. Herd selection criteria included factors such as having not been vaccinated, availability of blood samples, and willingness to participate. Conducting a small survey of excluded herds may help assess differences and adjust interpretations. In total, three dairy herds from each region were selected based on the above criteria, and a random simple sampling approach was then used to obtain at least 100 animals from each farm. Duplicate samples from the same animal were identified and removed by unique earmark identifiers. Due to data availability limitations, there was no pre-selection process for candidate covariates, and only age (parity) information was available and included in the analysis. Blood samples (approximately 5 ml) were collected from each animal using disposable needles and plain blood tubes via coccygeal vein venipuncture. The samples were properly labeled, and transported to the Henan Dairy Herd Improvement (Henan DHI) Centre in Zhengzhou within 24 hours in an ice box. The serum samples were separated by centrifugation at 3000 rpm for 10 minutes and stored at -20 ℃ for a maximum period of 12 months.

The sample size calculation was performed using the PropMisclassSampleSize software (Version 6.0, available at http://www.medicine.mcgill.ca/epidemiology/Joseph), following the detailed procedure outlined in previous reports (Dendukuri et al., 2010; Dendukuri et al., 2004). We adopted the average coverage criterion, a highest posterior density (HPD) length of 0.15

and a 95% HPD coverage, and uniform prior information for prevalence and informative priors for the *Se* and *Sp* of these tests (Table 3.1). These prior information and assumptions resulted in a minimum sample size of 502 total from two populations. Due to the limitation of this calculation method to independence between tests and a maximum of three tests (Dendukuri et al., 2004), we manually increased the final sample size (n = 603) by 20% to adjust for potential dependencies between tests as the rationales of these tests are based on the detection of antibodies against *Brucella* spp. Considering possible sample loss and insufficient volume during the sampling and testing process, the final number of samples collected was 651.

Table 3. 1 Prior distributions used for sensitivity, specificity, and prevalence estimates in the analysis of the sensitivity and specificity of four serological tests for the detection of antibodies against *Brucella* spp. in dairy cattle

Priors	Descriptions (mode + 95% of certainty of lower/upper limit)	Parameters [c]	References
RBT - Sensitivity	0.812 (0.60)	B(13.58, 3.91)	Gall et al. (2004)
RBT - Specificity	0.863 (0.60)	B(9.92, 2.42)	Gall et al. (2004)
SATc[i] - Sensitivity	0.759 (0.60)	B(21.40, 7.48)	Gall et al. (2004)
SATc[i] - Specificity	0.863 (0.60)	B(9.92, 4.42)	Gall et al. (2004)
PR - Prevalence	0.020 (0.20)	B(1.30, 15.80)	Liu et al. (2020a)
ZR - Prevalence	0.080 (0.01)	B(1.32, 3.67)	Wan (2017)
$\mu_{\text{C-ELISA, D-}}$	expected range (0.1 to 0.35 PI) [a]	U(-2.3, -0.7)	Kit instruction
$\mu_{\text{C-ELISA, D+}}$	expected range (0.4 to 1.0 PI) [a]	U($\mu_{\text{C-ELISA,D-}}$, 0.0)	Kit instruction
$\tau_{\text{C-ELISA, D-}}$	Diffuse distribution [a]	G(0.01, 0.01)	
$\tau_{\text{C-ELISA, D+}}$	Diffuse distribution [a]	G(0.01, 0.01)	
$\mu_{\text{FPA, D-}}$	expected range (75 to 95 mP) [b]	U(-0.3, 0.0)	Kit instruction
$\mu_{\text{FPA, D+}}$	expected range (120 to 180 mP) [b]	U($\mu_{\text{FPA, D-}}$, 0.6)	Kit instruction
$\tau_{\text{FPA, D-}}$	Diffuse distribution	G(0.01, 0.01)	
$\tau_{\text{FPA, D+}}$	Diffuse distribution	G(0.01, 0.01)	
β_{parity}	Diffuse distribution	N(0, 0.01)	
σ_{farm}	Diffuse distribution	U(0, 1)	

[a]: log-transformation; [b]: log-transformation of 0.01 times mP; [c]: B means Beta distribution; U means Uniform distribution; G means Gamma distribution; Beta distribution calculation: having a 95% confidence that estimated value is more/less than the lower/upper limit at a mode.

3.3.2 Diagnostic tests and latent condition

3.3.2.1 Definition of the latent condition

RBT, SAT, FPA, and C-ELISA were performed on each sample. The testing procedures were performed in a blinded manner, with identifiable numbers removed from the samples, and the results were kept confidential until all tests were completed. The rationale of these tests is based on detecting antibodies against *Brucella* spp., with RBT, C-ELISA, and FPA tests primarily targeting IgG1 antibodies (along with some IgM and IgA) (Ducrotoy et al., 2016; Rahman et al., 2019). SAT primarily detects IgM antibodies, while also capable of detecting some IgG and IgA antibodies. The latent condition modeled jointly by the four tests is determined by the overlap of the biomarker distributions detected by each test in the host. In our case, the latent condition pertains to *Brucella* spp. seropositivity rather than specific antibody type or active infection. It is worth noting that *Brucella* spp. has been reported to elicit the production of lifelong antibodies in cattle (Bercovich, 1998). Therefore, our hypothesis posits that antibodies will persist throughout the lifetime of cattle after infection with *Brucella* spp. However, it is possible that some animals exposed to *Brucella* spp. may not produce detectable antibody titers, and thus, they might not be captured by this latent condition. These animals constitute a fraction of the exposed population that our analysis cannot account for. A diagram illustrating the latent condition is presented in Figure 3.2, adapted from Ducrotoy et al. (2016); Marquetoux et al. (2023). Acute *Brucella* infection in dairy cattle occurs shortly after exposure, characterized by high bacterial sheddings, clinical signs like fever and abortion, and strong serological responses. Chronic infection develops when the bacteria persist, often asymptomatically, with intermittent shedding and lower antibody levels.

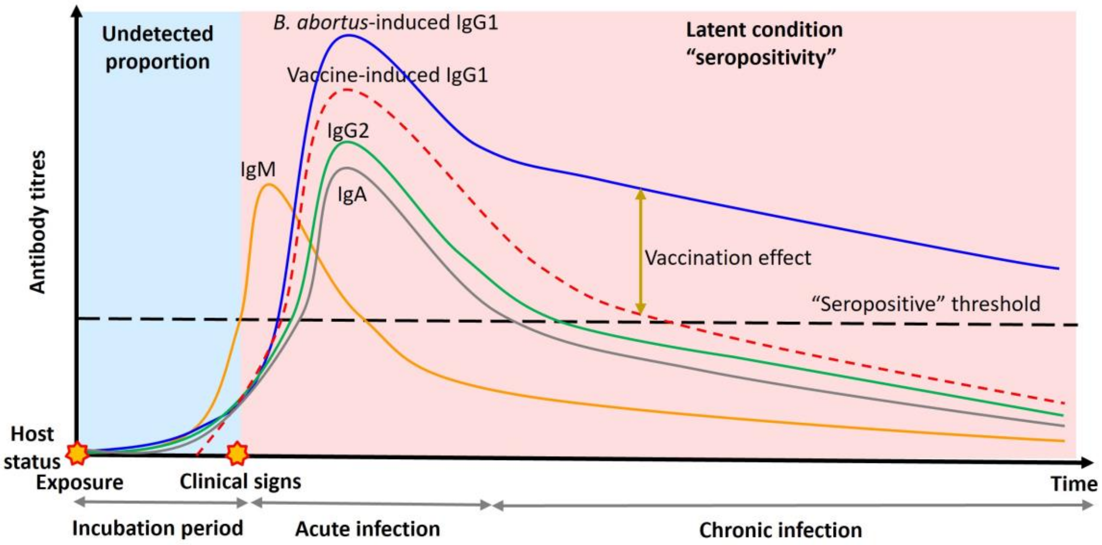

Figure 3. 2 A diagram illustration of *Brucella* spp. physiopathology and possible dynamics of biomarkers on specific antibodies, with a corresponding latent condition for BLCM. Note: The stars indicate the time points of exposure and manifestation of clinical signs.

3.3.2.2 Rose Bengal test (RBT)

RBT was performed as recommended by the World Organisation for Animal Health (WOAH, 2023b). Briefly, all sera, antigen, and positive and negative controls were brought to room temperature at least 30 minutes in advance. A 25 µl of RBT antigen and the same volume of serum sample were added to a transparent glass slide and mixed thoroughly. The reaction was observed within 4 minutes post-mixing as positive if agglutination was observed and negative if there was no reaction between serum and antigen. If a doubtful agglutination occurred, the sample was retested. The antigen, positive, and negative controls were purchased from the Harbin Pharmaceutical Group Bio-vaccine Co., Ltd, China.

3.3.2.3 Standard Agglutination Test (SAT)

SAT was performed following the protocols outlined in the Chinese National Standard Diagnostic Techniques for Animal Brucellosis (GB/T 18646-2018) (Li et al., 2021). The SAT antigen, positive, and negative controls were purchased from the National Animal Brucellosis Reference Laboratory at the China Institute of Veterinary Drug Control. All sera, antigen, and positive and negative controls were brought to room temperature 30 minutes in advance. Sera were subjected to serial 2-fold dilutions using normal saline containing 0.5% carbolic acid, ranging from 1:25 to 1:200. 100 µl of diluted serum was thoroughly mixed with 100 µl SAT antigen solution, resulting in a final serum dilution range of 1:50 to 1:400. The reaction mixture was sealed and placed in a 37 °C incubator for 18-24 hours. Interpretation of the results followed the criteria established by the Chinese National Standard Diagnostic Techniques for Animal Brucellosis (GB/T 18646-2018) (Li et al., 2021). Sera with endpoint titers of ≤ 1:50 were classified as seronegative, while titers of ≥ 1:100 were considered positive.

3.3.2.4 Fluorescence Polarization Assay (FPA)

FPA was performed following the instructions provided in the kit and as described elsewhere (Higgins et al., 2017). The FPA kit was purchased from Ellie LLC, USA. Briefly, all reagents and samples were allowed to return to room temperature for at least 30 minutes. 20 µl of serum sample was added to a glass tube containing 1 ml of dilution solution. The mixture was thoroughly mixed and allowed to stand for 2-5 minutes at room temperature. An initial reading was obtained on a fluorescence polarisation analyzer (FPM, portable Sentry 300 instrument, Ellie LLC, USA) to measure the light scatter value. Next, 10 µl of antigen labeled with fluorescein isothiocyanate was added to the glass tube and mixed thoroughly. After standing for an additional 2-5 minutes, a second reading was taken using the FPM. The final reading, expressed in millipolarisation units (mP), had to fall within the range of 70-95 mP units for negative control and 120-250 mP units for positive control, as specified by the kit instruction. The FPA results were interpreted twice independently, respectively (1) using the kit-provided cut-off value where samples were judged as positive if a mP was ≥ 100 mP and negative if < 100 mP; (2) employing the optimized cut-off value provided by BLCM. This involved subjecting the continuous values (mP) to a subsequent Bayesian Receiver Operating Characteristic (ROC) analysis that maximized the sum of *Se* and *Sp*. The cut-off values could be adjusted according to intended use, but the optimal cut-off value balances *Se* and *Sp* to reduce the relative consequences of misclassification.

3.3.2.5 Competitive enzyme-linked immunosorbent assay (C-ELISA)

The C-ELISA was performed with a *Brucella* Compac 2.0 C-ELISA kit purchased from INGEZIM, Spain using the procedure described by the manufacturer and other studies (Zeng et al., 2017; Zhu et al., 2020). Percent inhibition (PI) was calculated to measure the antibody titers by using the formula suggested by the manufacturer of the kit:

$$PI = \left[1 - \left(\frac{Optical\ density\ value\ of\ each\ test\ sample}{Mean\ optical\ density\ value\ of\ negative\ control}\right)\right] \times 100\%$$

The C-ELISA results were interpreted twice independently, (1) using the kit-provided cut-off value where samples were judged as positive if PIs were ≥ 40%, and negative if PI (%) < 40%; (2) employing the optimized cut-off value provided by BLCM. This involved subjecting the continuous values (PI) to a subsequent Bayesian Receiver Operating Characteristic (ROC) analysis that maximized the sum of *Se* and *Sp*.

3.3.3 Statistical analyses

3.3.3.1 Apparent test results

Following the cut-off values provided by kit manufacturers, we first dichotomized the raw test results of FPA and C-ELISA into binary outcomes to calculate the apparent prevalence. The apparent prevalence obtained by each serological test was calculated for each region with the corresponding 95% confidence interval (CI) using Wilson binomial confidence limits. The kappa statistics and its 95% CI were estimated among four serological tests. These calculations were achieved through the ‘epiR’ package (version: 2.0.65) (Stevenson et al., 2022).

3.3.3.2 Bayesian Latent Class Model (BLCM) framework

We adopted a “two binary tests and two continuous tests in two populations” approach, with each region treated as an independent population, namely PR and ZR. Since the detection rationale of four tests is grounded in the same biological principle of detection of antibodies against *Brucella* spp. (Figure 3.2), correlations between tests exist and need to be accounted for in the diagnostic evaluation (Johnson et al., 2019). Therefore, the four serological tests under evaluation were assumed to be conditionally dependent, given the true disease status.

The outcomes of RBT and SAT were considered dichotomous, whereas the FPA and C-ELISA outcomes were treated as continuous. FPA (mP) results were log-transformed with 0.01 times the original value, and C-ELISA (PI) result values were log-transformed to achieve approximate normality in the analysis and converted back in the presentation of the results (Choi et al., 2006). Bayesian ROC curves were created to estimate the optimal cut-off values by maximizing the Youden Index (sum of *Se* and *Sp*) (Youden, 1950). The Bayesian ROC analysis procedure in the absence of a gold standard has been developed and outlined in previous reports (Choi et al., 2006; Jones et al., 2009; Yang et al., 2022b).

In BLCM, the true disease status of individual cattle ($z_{[i]}$) was modeled using a Bernoulli sampling with the probability of being seropositive. A hierarchical model structure was adopted to estimate the probability of *Brucella* spp. seropositivity ($P_{[i]}$) by incorporating fixed effects for region and age, and a random effect for farm (Yang et al., 2022a). The true disease status ($z_{[i]}$) was then incorporated into Bernoulli sampling for both RBT and SAT results. More specifically, the apparent RBT results depended on Se_{RBT}, Sp_{RBT}, and true disease status ($z_{[i]}$). In the next step, SAT conditional *Se* and *Sp* were separately modeled as $Se_{\mathrm{SATc}[i]}$ and $Sp_{\mathrm{SATc}[i]}$ depending on RBT outcomes (i = 1 or 2, 1 means RBT- or 2 means RBT+), which could be expressed as $Se_{\mathrm{SATc}[1]} = Se_{\mathrm{SAT}}|\mathrm{D+,RBT-}$, $Se_{\mathrm{SATc}[2]} = Se_{\mathrm{SAT}}|\mathrm{D+,RBT+}$, $Sp_{\mathrm{SATc}[1]} = Sp_{\mathrm{SAT}}|\mathrm{D-,RBT-}$, $Sp_{\mathrm{SATc}[2]} = Sp_{\mathrm{SAT}}|\mathrm{D-,RBT+}$. If SAT is independent of RBT, then SAT conditional *Se* should remain constant regardless of the outcome of RBT; if they are dependent, then the SAT conditional *Se* should change given the outcome of RBT. Furthermore, Gaussian mixture models were used to model for log-transformed FPA and C-ELISA outcomes, with separate mean (μ) and precision (τ, inverse of the variance = $1/\sigma^2$) parameters depending on the true disease status (z_i) and RBT, and SAT outcomes (Choi et al., 2006; Coupe, 2021; Yang et al., 2022a). Random correlation coefficients were incorporated to account for the dependence between FPA and C-ELISA. Given the true disease status, the correlation coefficients between binary tests were calculated based on *Se* and *Sp* of RBT and SAT. The correlation coefficients between binary tests (RBT & SAT) and continuous tests (FPA & C-ELISA) were determined using a mixture of mean and variance of continuous tests, along with *Se* and *Sp* of binary tests, conditional on the true disease status. This methodology was initially introduced by Coupe (2021). The reproducible R code for the model was provided at https://github.com/YuWang1009/BLCM.git.

3.3.3.3 Definition of prior distributions

The model assuming conditional dependence was over-parameterized because it had more parameters than the number of degrees of freedom in the data, and hence required prior information to be elicited for disease prevalence, *Se* and *Sp* of RBT and SAT, and parameters related to the Gaussian mixture model to achieve model identifiability (Cheung et al., 2021; Jones et al., 2010). The prior distributions used in the model are outlined in Table 3.1. To elicit prior information, beta distributions were used for prevalence (π), Se_{RBT}, Sp_{RBT}, $Se_{\mathrm{SAT(RBT-)}}$, $Sp_{\mathrm{SAT(RBT+)}}$, $Se_{\mathrm{SAT(RBT-)}}$, $Sp_{\mathrm{SAT(RBT+)}}$. These prior distributions were based on published reports on cattle or previous test results (Gall et al., 2004; Liu et al., 2020a; Wan, 2017), independent of the current study population. For example, the *Se* estimate for RBT was assumed to be 0.812, with a 95% certainty that the estimate was greater than 0.60, generating a beta distribution Beta(13.58, 3.91). The *epi.betabuster* function from the 'epiR' package (version: 2.0.65) was used to generate parameters of the beta distribution (Stevenson et al., 2022). The choice of beta distribution for modeling *DSe*, *DSp*, and prevalence is due to its probabilistic nature (ranging between 0 and 1), flexibility in incorporating prior knowledge, and ease of Bayesian updating. Uniform priors were specified for the means of the log-transformed FPA and C-ELISA values for the non-diseased ($\mu_{FPA, D-}$, $\mu_{ELISA, D-}$) and diseased cattle ($\mu_{FPA, D+}$, $\mu_{ELISA, D+}$) based on possible maximum and minimum limits provided by kit suppliers. For example, the negative control of FPA must read between 75 mP and 95 mP, the corresponding log(0.01*mP) transformation ranged between -0.29 and -0.05. Therefore, we set the distribution of log-transformed mean FPA values as $\mu_{FPA, D-} \sim$ Uniform(-0.3, 0.0) to achieve sufficient diffusion. The prior distributions for parameters of means of log-transformed FPA and C-ELISA, conditioned on the true disease status, were derived in the same manner. It was assumed that diseased animals would exhibit higher mean values for FPA and C-ELISA than healthy animals to ensure model identifiability (Choi et al., 2006). The prior distributions were finally set as follows $\mu_{FPA, D-} \sim$ Uniform(-0.3, 0.0); $\mu_{FPA, D+} \sim$ Uniform($\mu_{FPA, D-}$, 0.6); $\mu_{ELISA, D-} \sim$ Uniform(-2.3, -0.7); $\mu_{ELISA, D+} \sim$ Uniform($\mu_{ELISA, D-}$, 0.3). Priors for $\mu_{FPA, D-}$, $\mu_{ELISA, D-}$, $\mu_{FPA, D+}$, and $\mu_{ELISA, D+}$ remained constant regardless of the RBT and SAT outcomes as there was no information about the mean values of FPA and C-ELISA, given RBT and SAT outcomes. Because individual animals were clustered within two regions, we added a farm-level random effect and assumed it to be normally distributed, which can be modeled using a logit-normal distribution with additional covariates (prevalence by region, parity). Diffuse prior distributions, such as $\tau \sim$ Gamma(0.01, 0.01), $\beta_{parity} \sim$ Normal(0.0, 0.1), $\sigma_{farm} \sim$ Uniform(0, 1), were specified for the precision of normal distribution of FPA and ELISA results, parity effect and farm-level random effect. The region covariate coefficient parameter was shown as $alpha_i$ (i = 1, 2), which was obtained by logittransforming region-specific prevalence based on previous reports (Liu et al., 2020a; Wan, 2017).

3.3.3.4 Sensitivity analysis on the prior distribution models

Scenario 1 was presented as the primary model in which prior information was incorporated. Additionally, we changed prior distributions one by one to conduct a sensitivity analysis, intending to explore the effects of prior choices on posterior estimates for animal-level prevalence, *Se*, and *Sp*. A flat prior (Beta (1, 1)) distribution was used in the sensitivity analysis to replace elicited priors for prevalence, *Se* and *Sp* of RBT and SAT, while other parameters (e.g., means and precision for FPA and C-ELISA, and standard deviation for farm random effect) remain constant across the additional seven scenarios given that they are diffuse prior distributions. The specific details of these scenarios can be found in Table S 3.1 of Appendix 2.

3.3.3.5 Model implementing and monitoring

Bayesian latent class analyses were implemented using JAGS software version 4.30 (Plummer, 2003) via the R package ‘R2jags’ (version: 0.7-1) (Su et al., 2021) in R version 4.2.1. Monte Carlo Markov Chain (MCMC) techniques were used to derive the full joint posterior distribution of parameters from prior distributions and test results. Two independent MCMC chains were run in parallel. For each chain, a burn-in period of 5,000 iterations was discarded; another 20,000 iterations were used for the posterior distribution. Regarding each parameter, a point estimate was defined as the median of its marginal posterior distribution, and a 95% credible interval was defined by the 2.5 and 97.5 percentiles of this marginal posterior distribution (termed 95% posterior credibility interval, 95% PCI). The convergence was checked by monitoring MCMC chain traces and autocorrelation plots, and computing Gelman and Rubin's statistics (Brooks et al., 1998).

3.3.4 Ethics approval

The study protocol was approved by a local ethics committee – Animal Experimental Ethical Inspection of Laboratory Animal Centre, Huazhong Agricultural University (Protocol Number: HZAUBU-2021-0003). The study involving human participants was reviewed and approved by peer review at Massey University Human Ethics Committee (Protocol Number: SOA 21/60). Written consent was obtained from farm owners before the collection of blood samples from dairy farms.

3.4 Results

3.4.1 Apparent test results

The apparent test prevalence for each region is presented in Table 3.2, with values ranging from 1.2% to 2.8% for PR, and 20.6% to 27.3% for ZR. The cross-classified counts, as shown in Table S 3.2 of Appendix 2 provide the frequency distribution of each of the two regions. Overall, 71 out of 651 samples tested positive on all four tests, 548 tested negative on all four tests, and 32 samples had 'differing' test results, indicating possible misclassification in the tests. The *kappa* agreement values between RBT & SAT, RBT & FPA, RBT & C-ELISA, SAT & FPA, SAT & C-ELISA, and FPA & C-ELISA were 0.847 (95% CI: 0.771, 0.923), 0.933 (95% CI: 0.893, 0.972), 0.910 (95% CI: 0.863, 0.957), 0.806 (95% CI: 0.736, 0.875), 0.872 (95% CI:

0.814, 0.930), 0.919 (95% CI: 0.875, 0.963), respectively, indicating there is good agreement among four tests.

Table 3. 2 Apparent test prevalence of detection of antibodies against *Brucella* spp. by each of four tests in PR and ZR regions

Test	Region	Apparent prevalence (%)	95% Confidence interval
RBT	PR	2.2	1.1, 4.4
	ZR	25.8	21.3, 30.7
SAT	PR	1.2	0.5, 3.2
	ZR	20.6	16.6, 25.3
FPA	PR	2.8	1.5, 5.2
	ZR	27.3	22.7, 32.3
C-ELISA	PR	2.5	1.3, 4.8
	ZR	24.2	19.9, 29.1

3.4.2 Determination of optimal cut-off values

The ROC curves for C-ELISA and FPA are depicted in Figure 3.3. Sensitivities and specificities of the FPA and C-ELISA were calculated at different cut-off values, ranging from 74 mP to 122 mP for FPA and 0.22 PI to 0.60 PI for C-ELISA. The FPA test demonstrated a slightly higher Youden index (ranging from 1.85 to 1.88) for cut-off values between 90.5 mP and 100.5 mP. The optimal cut-off value for FPA was 94.2 mP, resulting in *Se* of 89.9% (95% PCI: 84.3, 94.2) and *Sp* of 98.7% (95% PCI: 98.0, 99.3). At the kit-recommended cut-off, the FPA exhibited a *Se* of 86.1% (95% PCI: 79.1, 91.3) and *Sp* of 99.6% (95% PCI: 99.1, 99.9). C-ELISA test demonstrated a similar Youden index at around 1.78 for cut-off values between 0.37 and 0.44 PI. The optimal cut-off value of 0.403 PI was determined to maximize the Youden index (1.78, 95% PCI: 1.70, 1.85) for C-ELISA, resulting in a *Se* of 81.6% (95% PCI: 72.7, 88.3) and *Sp* of 97.1% (95% PCI: 96.0, 97.9). At the kit-recommended cut-off of 0.40 PI, the C-ELISA exhibited a *Se* of 81.8% (95% PCI: 72.9, 88.5) and *Sp* of 96.9% (95% PCI: 95.7, 97.8).

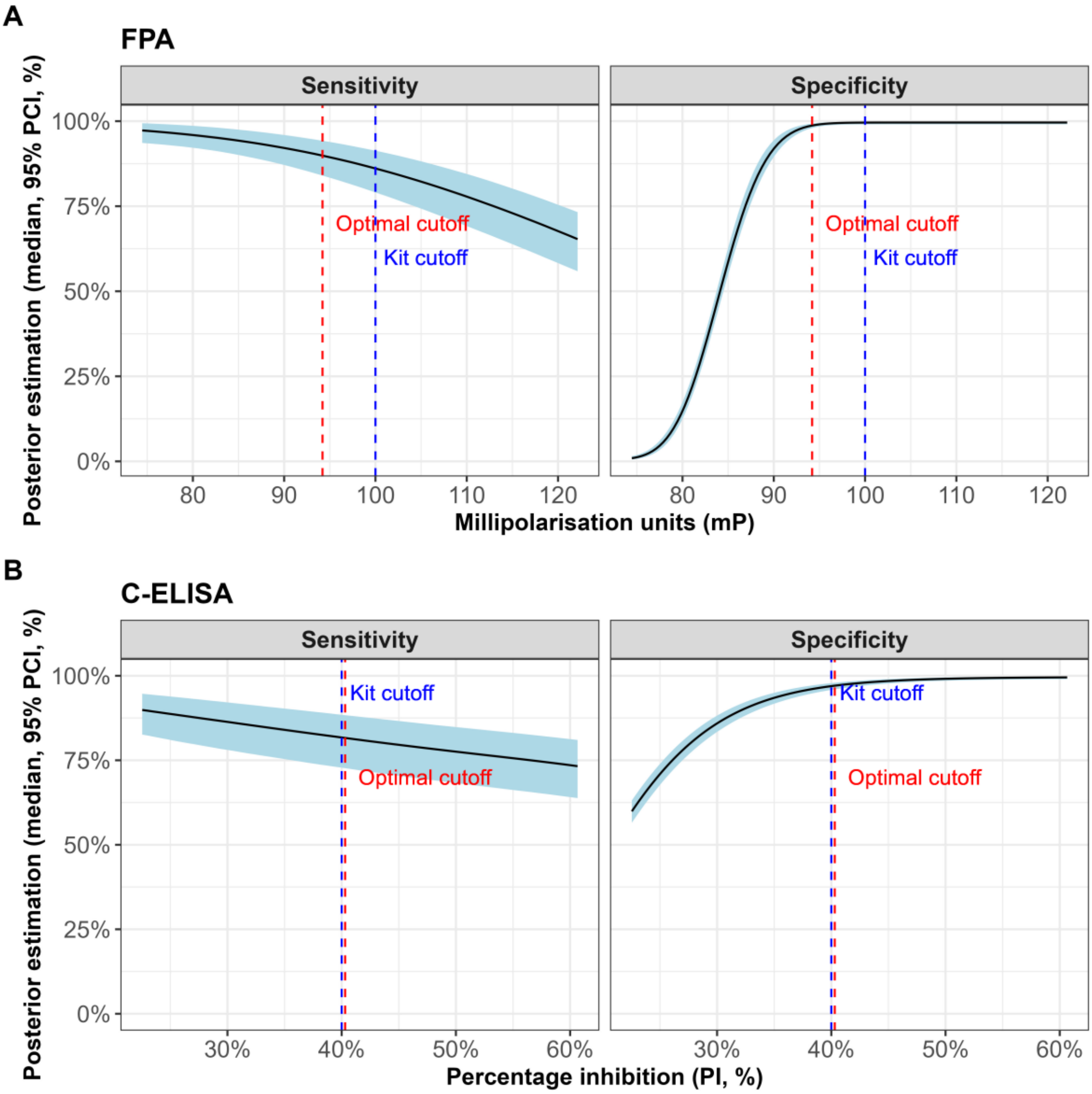

Figure 3. 3 Bayesian receiver operating characteristic (ROC) curves for the results of FPA (Panel A) and C-ELISA (Panel B) for detecting antibodies against *Brucella* spp. in dairy cows. The optimal cut-off value was determined by maximizing the Youden Index. Solid black line represents the median estimate with a 95% posterior credible interval (blue shade areas).

3.4.3 Sensitivity and specificity estimates of four tests

Table 3.3 shows the median of the posterior distribution along with 95% PCI for *Se* and *Sp* of the four tests for Scenario 1. This scenario represents the final model, wherein informative priors were incorporated. Model convergence was checked by plotting traceplots (See Figure S 3.1 in Appendix 2). Among the tests, FPA has the highest sensitivity (89.9% at optimal cut-off) and outperforms that of RBT (79.9%), SAT (69.7%), and C-ELISA (81.6% at optimal cut-off). All four tests also have good specificities, ranging from 97.1% to 99.6%. The posterior distributions of *Se* estimates of RBT and SAT are slightly shifted relative to the priors, whereas the posterior distributions of *Sp* of RBT and SAT are shifted to the right and highly concentrated (Figure S 3.2 in Appendix 2). Notably, the conditional sensitivity and specificity of SAT indicate a high dependence on the RBT test results. SAT has significantly higher *Se* in RBT-positive conditions ($Se_{\text{SATc[2]}}$ = 76.6%, 95% PCI: 68.6, 83.5) than in RBT-negative conditions ($Se_{\text{SATc[1]}}$ = 42.8%, 95% PCI: 28.1, 58.9), while the *Sp* of SAT is conversely higher in RBT-negative conditions ($Sp_{\text{SATc[1]}}$ = 99.6%, 95% PCI: 98.8, 99.9) than in RBT-positive conditions ($Sp_{\text{SATc[2]}}$ =82.1%, 95% PCI: 55.4, 96.4). We also find that the FPA test results are correlated with C-ELISA as evidenced by correlation coefficients (*rho*s varying from 0.30 to 0.78) (See Figure S 3.3 in Appendix 2). The binary tests (RBT and SAT) are correlated with continuous tests (FPA and C-ELISA) in the case of diseased animals (*rho*s varying from 0.33 to 0.51), but there is no correlation between them for non-diseased animals (See Figure S 3.3 in Appendix 2). The posterior distributions of the parameters used in the Gaussian mixture model are summarized in Table S 3.3 of Appendix 2.

3.4.4 Prevalence and parity effect

The estimated true animal-level prevalence of antibodies against *Brucella* spp. was found to be 4.7% (95% PCI: 4.0, 5.9) in PR and 30.3% (95% PCI: 28.5, 33.0) in ZR. Furthermore, the animal-level prevalence varied widely when examining specific farms, ranging from 0.9% to 37.4% (Table 3.3). The effect of parity on the detection of antibodies against *Brucella* spp. was assessed. The details about the distribution of individual parity for each farm and region were summarized in Table S 3.4 of Appendix 2. The results indicated that the odds of detecting these antibodies increased with parity, with estimates ranging from 1.2 to 2.1 for different parity groups compared to primiparous cows (Figure 3.4). The risk of *Brucella* positive in cows with 3rd parity was significantly higher than that in primiparous cows (OR = 2.1, 95% PCI: 1.5 – 2.8), but it was not significant in other groups.

3.4.5 Sensitivity analysis on priors

The sensitivity analysis conducted in an additional seven scenarios reveals the relative robustness of the model. When certain priors, such as prevalence, *Se*, and *Sp* of the RBT test, are changed to a flat distribution (Beta(1, 1)) in Scenarios 2, 3, and 5, the posterior estimates of animal-level prevalence, as well as the *Se* and *Sp* of the four tests, remain nearly identical to those obtained in Scenario 1 (fully informative priors). This indicates that the model is not heavily influenced by changes in these specific priors, as depicted in Figures 3.5 and 3.6. However, notable changes in the posterior estimates of animal-level prevalence, sensitivity, and specificity occur when the conditional *Se* and *Sp* of the SAT ($Se_{\mathrm{SATc[i]}}$ and $Sp_{\mathrm{SATc[i]}}$) are altered to a flat distribution. These changes are particularly prominent in the performance of the SAT, as demonstrated in Figures 3.5 and 3.6. This indicates that the model is sensitive to variations in the specified priors for SAT conditional *Se* and *Sp*.

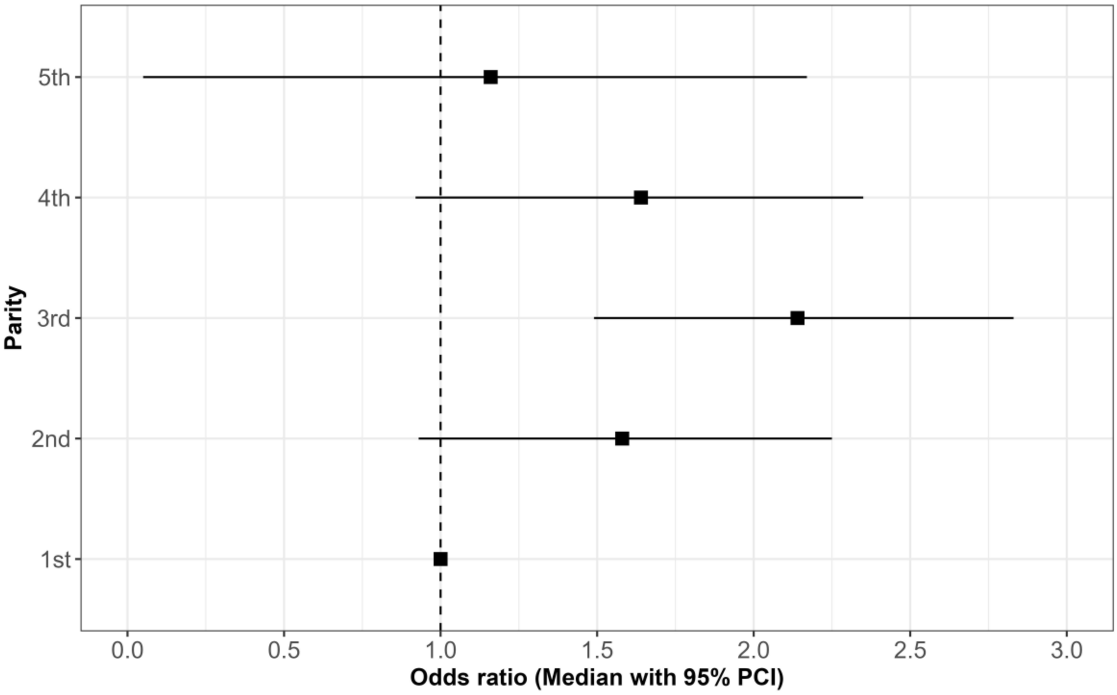

Figure 3. 4 Effects of parity (median, 95% posterior credibility interval - PCI) on the detection of antibodies against *Brucella* spp. in Henan Province

Table 3. 3 Sensitivity (*Se*) and specificity (*Sp*) estimates of the four tests and animal-level prevalence of antibodies against *Brucella* spp. of each region and farm with their 95% posterior credible intervals (PCIs) according to the model with informative priors

Parameters	Median (%)	95% PCI (%)
Se RBT	79.9	69.8, 87.5
Sp RBT	99.6	98.8, 99.9
Se SAT	69.7	61.4, 77.1
Sp SAT	99.5	98.7, 99.9
Se SAT $_{C[1]}$	42.8	28.1, 58.9
Sp SAT $_{C[1]}$	99.6	98.8, 99.9
Se SAT $_{C[2]}$	76.6	68.6, 83.5
Sp SAT $_{C[2]}$	82.1	55.4, 96.4
Se FPA (kit cut-off)	86.1	79.1, 91.3
Sp FPA (kit cut-off)	99.6	99.1, 99.9
Se FPA (optimal cut-off)	89.9	84.0, 94.2
Sp FPA (optimal cut-off)	98.7	98.0, 99.3
Se C-ELISA (kit cut-off)	81.8	72.9, 88.5
Sp C-ELISA (kit cut-off)	96.9	95.7, 97.8
Se C-ELISA (optimal cut-off)	81.6	72.7, 88.3
Sp C-ELISA (optimal cut-off)	97.1	96.0, 97.9
Prevalence PR	4.7	4.0, 5.9
Prevalence ZR	30.3	28.5, 33.0
Prevalence farm 1	0.9	0.9, 1.9
Prevalence farm 2	1.8	1.8, 4.5
Prevalence farm 3	10.7	9.7, 13.6
Prevalence farm 4	29.2	27.4, 32.7
Prevalence farm 5	24.5	21.8, 29.1
Prevalence farm 6	37.4	35.5, 40.2

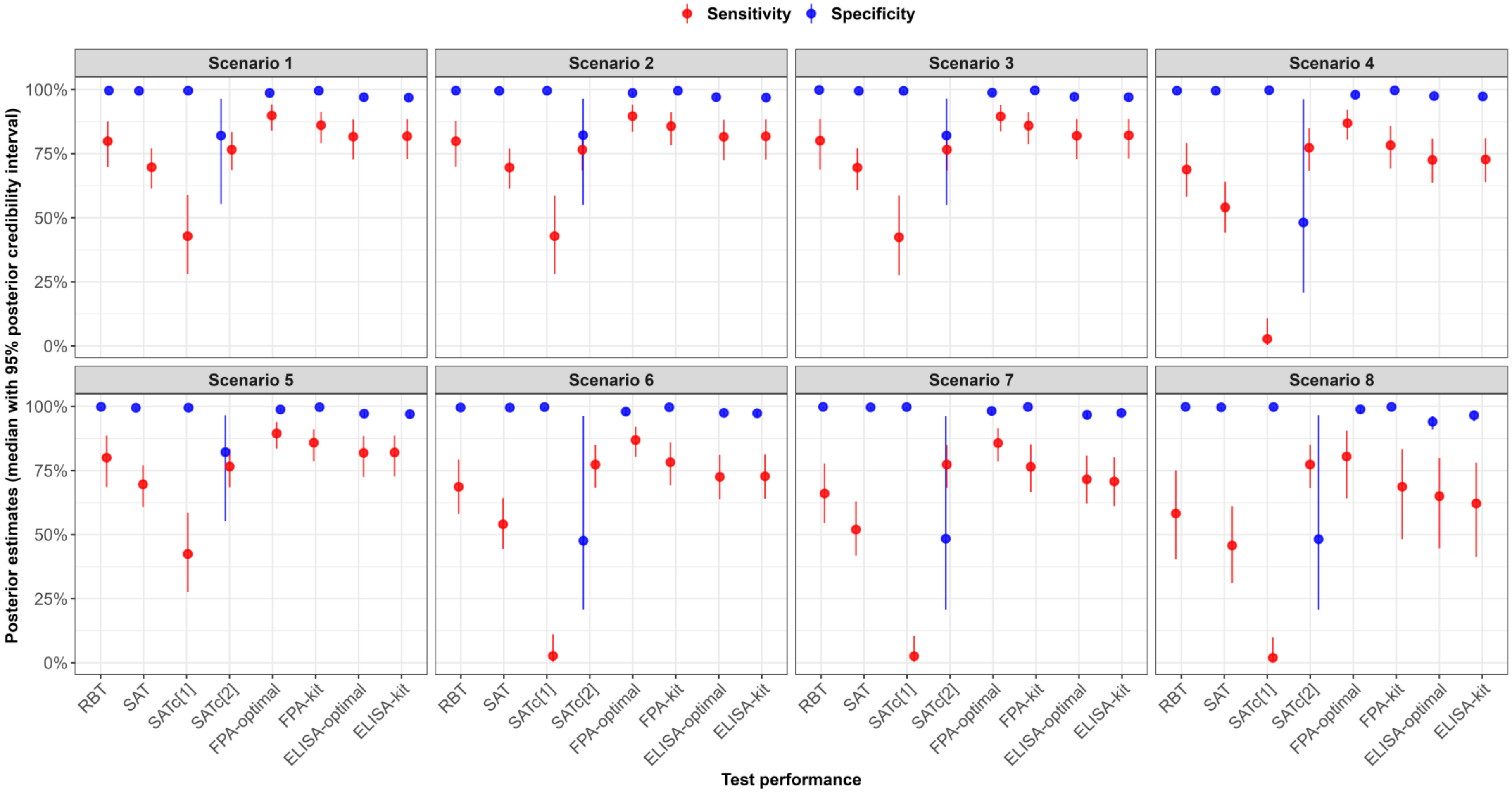

Figure 3. 5 A dot plot with bar (median, 95% posterior credibility interval - PCI) showing the impact of changing prior information on sensitivity and specificity estimates for eight scenarios. $_{C[1]}$: conditional on RBT-negative; $_{C[2]}$: conditional on RBT-positive

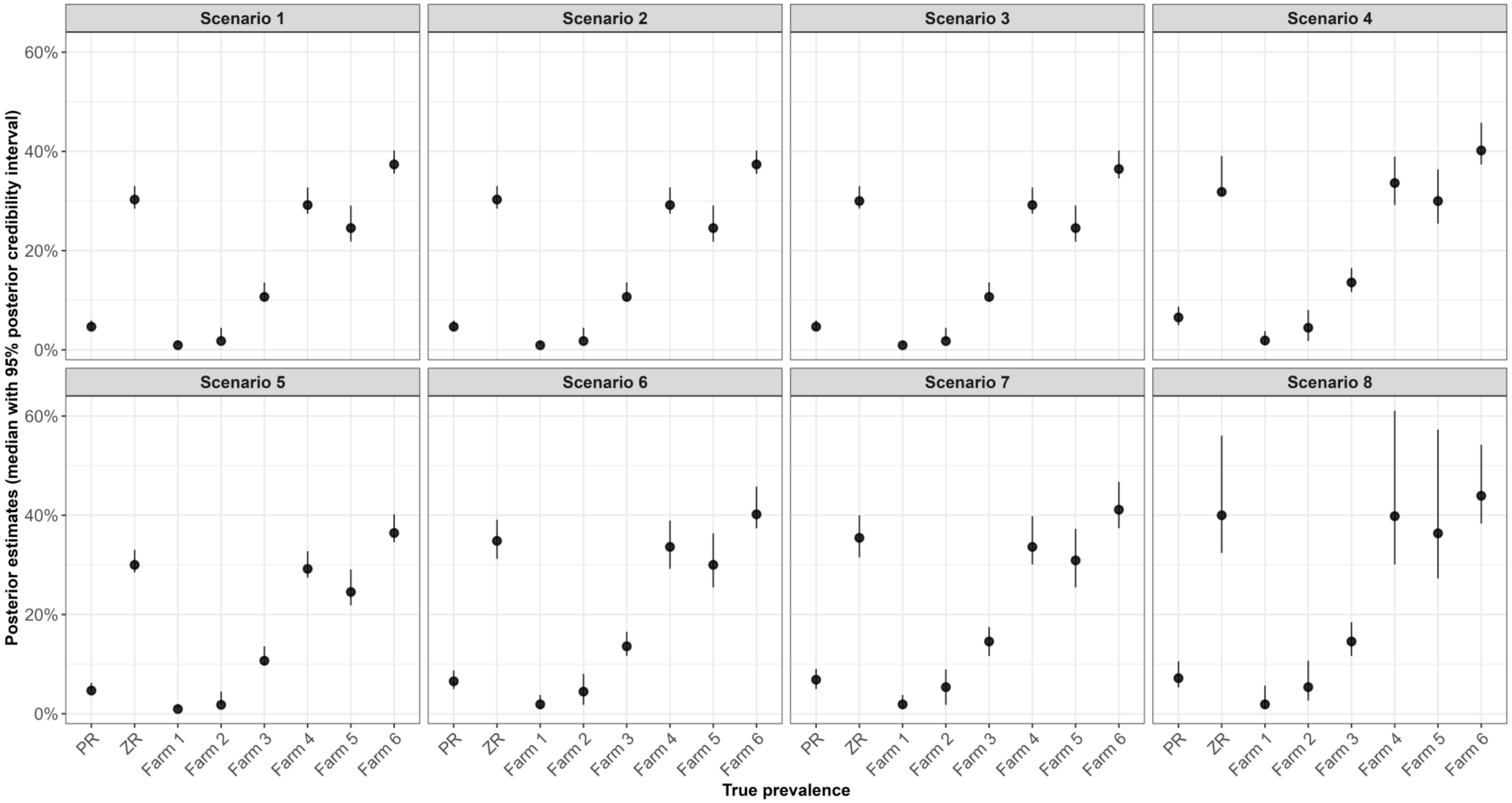

Figure 3. 6 A dot plot with bar (median, 95% posterior credibility interval - PCI) showing the impact of changing prior information on animal-level prevalence estimates within specific regions (PR and ZR) and farms for eight scenarios

3.5 Discussion

In this study we have successfully developed a novel BLCM that addresses the challenges of analyzing both continuous and discrete testing data with additional covariates, allowing us to estimate the optimal cut-off values of FPA and C-ELISA and assess the test performance of four serological tests for the diagnosis of *Brucella* spp. in Henan dairy farms. The developed BLCM framework offers remarkable flexibility and applicability in handling one or multiple continuous or discrete testing data, making it a valuable tool in diagnostic evaluation and prevalence estimation for animal or human diseases. The cut-off values, *Se*, and *Sp* estimates obtained from our model are essential information for various stakeholders, including farmers, decision-makers, and researchers, in the clinical diagnosis and screening of brucellosis. Furthermore, these findings can be utilized in quantitative risk assessments and prevalence estimations, providing a solid foundation for designing informed *Brucella* spp. control programs in Chinese dairy industries and indirectly benefiting human health.

3.5.1 Sensitivity and specificity estimates and their epidemiological implications

At the optimal cut-off value of 94.2 mP, our estimates for *Se* of 89.9% (95% PCI: 84.3, 94.2) and *Sp* of 98.7% (95% PCI: 98.0, 99.3) of FPA differ from a traditional validation study using a cut-off of 95 mP (relative *Se* = 98.6%, relative *Sp* = 100%) (Nielsen et al., 2001), particularly for *Se*. This may be because they used positive samples based on other tests (e.g., C-ELISA). Other studies conducted in Canada and Mexico indicated that FPA has 99.0% *Se* and 96.9%-99.9% *Sp* at 90 mP cut-off (Dajer et al., 1999; Nielsen et al., 1996). For C-ELISA, our sensitivity estimate (*Se* = 81.6%, 95% PCI: 72.7, 88.3) was lower than the previously reported estimates from a Bayesian latent class model (*Se* = 98.1%) (Wang et al., 2020c). This may be due to the difference in modeling approach, we used continuous data for modeling, while the previous study used binary data (Wang et al., 2020c). Another study conducted in Tanzania yielded a lower sensitivity estimate (*Se* = 62.3%) than this study for C-ELISA (Bodenham et al., 2021), which may be related to the unique study population and epidemiological context. The definition of 'optimal cut-off' used in this study is based on the balance of *Se* and *Sp* to minimize misclassification, but in practice, the 'optimal cut-off' value should depend on the applied scenario and purpose. In a high-prevalence setting, raising the cut-off value with a higher specificity may be crucial to reduce false positives and avoid unnecessary culling or interventions. Conversely, in a low-prevalence or eradication phase, lowering a cut-off value with higher sensitivity is preferred to minimize false negatives and prevent the persistence of latent infection.

The *Se*/*Sp* ratio can provide insights into the overall test performance of tests, particularly when balancing the trade-off between *Se* and *Sp*, but does not provide a complete picture on its own. Depending on the specific epidemiological question and context, *Se* or *Sp* may be prioritized. In high-prevalence farms, high sensitivity improves the accurate detection of infected animals, reducing transmission risk. In low-prevalence farms, high specificity avoids unnecessary measures for healthy animals but may have limited impact due to the small proportion of true positives. Additional measures might be needed in low-prevalence populations to optimize disease control efforts. However, tests with high specificity are commonly employed as a 'confirmatory' test following a positive RBT result. This diagnostic practice has been commonly used in dairy cattle and human studies in China and is also recommended by the Chinese National Standard Diagnostic Techniques for Animal Brucellosis (GB/T 18646-2018) (Li et al., 2021). This practice has been argued that the concept of a 'confirmatory' test is not absolute and can be misleading (Ducrotoy et al., 2018). In the absence of vaccination and a high-prevalence context, a positive RBT does not require further retesting by a 'confirmatory' test such as SAT and ELISAs as the *Se*/*Sp* ratios of others are not better than that of RBT, like in ZR region. It is in the context of vaccination that SAT or C-ELISA becomes the 'confirmatory' test for positive RBT results, aiming to mitigate the false-positive problems associated with vaccinal antibodies and unnecessary culling, as advocated by WOAH rules (WOAH, 2023b). Samples from non-vaccinated and high-prevalence areas such as ZR may be exempt from 'confirmatory' test if they are positive on screening test (e.g., RBT) given the high specificities assessed above (all > 97%), but if samples are from vaccinated or low-prevalence areas like PR 'confirmatory' tests should be used to mitigate the problem of false positives, especially when the true prevalence is close to or below 1-*Sp*. When farms are at the last stage of the brucellosis elimination program with a low prevalence (e.g., <1%), testing in series may be a priority to identify all positive animals, rather than considering the cost. Other factors, such as cost, availability, and ease of use, should also be considered when selecting a test for use in epidemiological studies or clinical practice.

As the BLCM iterated through the observed data, we found interesting shifts in the posterior distributions of *Se* and *Sp* of RBT and SAT (See Figure S 3.2 in Appendix 2). Specifically, the posterior distribution of *Se* of RBT and SAT experienced a marginal shift, suggesting that our initial assumptions about this parameter were relatively robust and aligned well with the empirical data. Conversely, the posterior distribution of *Sp* of RBT and SAT exhibited a notable shift towards higher values, which supported a higher *Sp* than the original assumptions. This adjustment highlights the profound impact of empirical evidence in refining our understanding of test performance. These shifts in the posterior distributions of *Se* and *Sp* provide crucial insights into the performance characteristics of the diagnostic tests. Similar shifts in the posterior distribution of parameters have been found in many studies (Arif et al., 2018; Rahman et al., 2019) as prior assumptions can only provide certain information and cannot perfectly fit the empirical data.

To the best of our knowledge, the *Se* and *Sp* of four serological tests may vary depending on factors such as parity and other relevant factors, such as species, stage of infection, and sample type (Bodenham et al., 2021; Ducrotoy et al., 2016). The immune response can differ among animals of different ages due to variations in immune maturity and previous exposure to *Brucella* spp. Older cows that have been previously exposed to *Brucella* spp. may have a higher likelihood of developing detectable antibody titers, while younger animals, particularly those that have not yet been exposed to the bacteria or are in the incubation stage before the occurrence of abortion, may not have reached detectable levels of antibodies (Ducrotoy et al., 2016; Ducrotoy et al., 2018). This age-related difference can lead to higher sensitivity in older animals but lower sensitivity in younger animals. In this study, we integrated parity as a proxy for age into our Bayesian model to obtain a posterior marginal distribution of *Se*, *Sp,* and parity-specific odds ratios. This approach simplifies the model and facilitates the practical application and generalization of the posterior estimates. On the other hand, the *Sp* estimates of serological tests are generally high, but false-positive cases can still occur due to cross-reactivity with other bacteria, such as *Yersinia enterocolitica* or *Escherichia coli* (Ducrotoy et al., 2016; Ducrotoy et al., 2018; Jones et al., 2010; Jones et al., 2009). The *Sp* estimates may be overestimated in the presence of concurrent health conditions caused by these pathogens. The choice of serological tests for diagnosis and screening of *Brucella* spp. should be based on a comprehensive understanding of the population under investigation, the existence of cross-reaction pathogens, the specific objectives of the testing, plausible prevalence, and performance characteristics. Interpreting test results in conjunction with other available clinical and epidemiological information contributes to an accurate classification based on these tests.

3.5.2 Optimal cut-off values for FPA and C-ELISA

Our BLCM in Henan dairy populations shows that the optimal cut-off values for the FPA and C-ELISA kits are 94.2 mP and 0.403 PI respectively. The cut-off value for FPA is lower than the kit-recommended while that for C-ELISA is almost consistent with the recommendation (Figure 3.3). Our model indicates that lowering the cut-off value for FPA leads to improved *Se* estimates, while the decrease in *Sp* estimates is negligible. This observation is supported by the minimal change observed in the Youden Indexes for FPA within the range of 90.5-100.5 mP. However, it is important to note that test results falling within this range should be regarded as suspicious and controversial. In such cases, we recommend retesting these animals with another supplementary test with high specificity to enhance diagnostic discrimination (WOAH, 2023b).

Alternatively, if a decision is made solely based on a single test result falling within this range, the interpretation of test results should take into account the purpose of testing, the likely prevalence and/or pre-test likelihood of disease, and economic and public health consequences of misclassification of disease status. For example, in the case of introducing animals to a *Brucella*-free farm, *Se* may be prioritized over *Sp* to minimize the chance of misclassifying positive animals as negative by adjusting to a lower cut-off value. This consideration is crucial during an emerging outbreak, as the potential cost of false negatives is typically higher than that of false positives.

Comparing the current study with other reports, researchers have reported various optimal cut-off values for FPA and C-ELISA tests based on maximizing the Youden index, which is the most commonly used criterion to strike a balance between sensitivity and specificity in ROC analysis (Greiner et al., 2000c). For instance, Ren et al. (2016) reported a cut-off value of 95 mP based on ROC analysis, while many studies used the kit-recommended cut-offs (Eurofins-INGENASA, 2022). However, it is important to note that cut-off values can be adjusted for various reasons. The relatively higher cut-off value used by other studies may improve the *Sp* and thus are suitable for use in low prevalence and test-and-slaughter contexts to reduce false positive misclassifications from financial considerations (Greiner et al., 2000a). In our study, an optimal cut-off value of FPA lower than that recommended by the kit could improve *Se* with a negligible sacrifice of *Sp*. Another interesting point is that in the Youden index calculation, adjusting the cut-off values of both FPA and C-ELISA within certain ranges (74-122 mP for FPA, 0.22-0.60 PI for C-ELISA) has a weighted effect on *Se* than on *Sp* (Figure 3.3). We assume that this may be related to the nature of *Brucella* spp. and the high specificity of serological tests. Therefore, we recommend the need for validation of cut-off values when testing in new populations or species, especially if the prevailing *Brucella* species and the animal species housed differ from those previously evaluated. The choice of cut-off values should be based on empirical evidence, considering the purpose of testing, the specific epidemiological context, and potential implications on result interpretation to ensure the accuracy and reliability of the tests (Greiner et al., 2000b). The range of possible cut-off values provided by our model (see Figure 3.3), along with the associated *Se* and *Sp* for FPA and C-ELISA, offers users the flexibility to adjust cut-off values according to their specific requirements and objectives, taking into account factors such as economic justifications, transmission risks, and public health implications.

3.5.3 Justification of Bayesian Latent Class Model

Given the absence of a gold standard test, Bayesian latent class approaches have been developed to evaluate the test performance of one or multiple test(s), to determine optimal cut-off values for continuous test outcomes, and to estimate population prevalence along covariate effects. Using the kit-recommended cut-off values, we found that 4.9% (32/651) of samples showed differences in test results, indicating possible misclassifications. The BLCM treated the true disease status as a latent (unobserved) variable and incorporated multiple test results and prior information to adjust for test imperfections and derive more accurate estimates of *Se* and *Sp*. These approaches are considered the most appropriate for obtaining unbiased estimates when evaluating tests without a gold standard (Cheung et al., 2021; Gardner et al., 2010; Johnson et al., 2019; Yang et al., 2022b). Following the STARD-BLCM (Kostoulas et al., 2017), careful consideration should be given to the definition of the latent class when interpreting the results. In our study, we used four serological tests to detect *Brucella*-specific antibodies, so the latent class estimated in this study was the seropositivity of *Brucella*-specific antibodies instead of *Brucella* spp. itself. Given that the diagnostic tests used in this study cannot differentiate between different *Brucella* species, one underlying assumption is that *B. abortus* is the predominant species in dairy herds in Henan Province (Wang et al., 2024b), but this requires further confirmation by bacterial isolation and identification. Additionally, we did not intend to explore more fundamental correlations between IgM and IgG status, as all the tests under evaluation were unable to differentiate between specific types of antibodies. Considering the nature of *Brucella* spp. infection, which is typically chronic and characterized by persistent antibodies (Bercovich, 1998; Ducrotoy et al., 2016; Rahman et al., 2019), it is reasonable to assume that modeling the presence of antibodies closely represents the optimal latent class of *Brucella* spp. infection (Rahman et al., 2019). Nevertheless, some animals may be in the incubation period (e.g., before the occurrence of the first abortion) or immunocompromised, resulting in undetectable levels of antibodies (Ducrotoy et al., 2016), so the true *Se* of the four tests may be lower than the current estimates in these animals. Limmathurotsakul et al. (2010) defined the latent class as the true infection status in their latent class model with the addition of culture, which differed from our latent class definition (presence of specific antibodies) (Arif et al., 2018; Rahman et al., 2019; Wang et al., 2020c), so their *Se* and *Sp* estimates may be more accurate as discussed before. Prospective research endeavors could incorporate bacterial culture and PCR tests to address this knowledge gap and explore the potential relationship between bacterial shedding and antibody dynamics.

Existing studies have primarily focused on utilizing either binary test results (Arif et al., 2018; Rahman et al., 2019; Wang et al., 2020c) or continuous test results (Choi et al., 2006; Jones et al., 2009; Yang et al., 2022b), with limited research exploring the integration of both binary and continuous data within a Bayesian latent class model. In our study, we built upon the work of Coupe (2021), who used two binary assays and one continuous assay to detect specific antibodies against *Toxoplasma gondii* in a single population. We expanded upon this framework by incorporating two binary tests and two continuous tests across two distinct populations. One of the major strengths of our model lies in its ability to seamlessly integrate discrete and continuous testing data within a Bayesian framework without any loss of information, all in a single step. A recent study used the posterior positive probability, conditional on *Se*, *Sp*, and prevalence estimates, to determine the cut-off value that maximized the sum of sensitivity and specificity for ELISA (Olsen et al., 2022). Although this approach avoids data loss due to dichotomization, it requires multiple steps to obtain the final posterior estimates (Olsen et al., 2022). Furthermore, additional covariates can also be incorporated into the model to adjust the effect of host characteristics on the cut-off, *Se* and *Sp*.

3.5.4 Prevalence estimates

The overall apparent seroprevalence in the PR and ZR regions varied across the four tests, ranging from 1.2% to 2.8% in PR and 20.6% to 27.3% in ZR by four serological tests. This disparity reflects the distinct prevalence of antibodies against *Brucella* spp. in these two dairy populations. However, the apparent seroprevalence in previous studies was 2.2% and 7.8% in PR and ZR, respectively (Liu et al., 2020a; Wan, 2017). Several factors may account for these discrepancies, such as variations in sample size and the characteristics of the sampled populations. Only six farms were included in this study, which may limit the representativeness compared to the previously reported studies (Liu et al., 2020a; Wan, 2017). Additionally, the animal-level prevalence in our study was estimated by adjusting for the *Se* and *Sp* of four evaluated tests, parity, and farm random effects, which were not accounted for in the aforementioned reports (Liu et al., 2020a; Wan, 2017). The potential rapid transmission of *Brucella* spp. in sampled farms may also occur in the gap between investigations, leading to an increase in prevalence. In this study, the posterior distributions of prevalence in two regions were shifted to the right, which may be related to the relatively conservative and diffuse prior distributions and be driven by observed test results. This alteration in the distribution of prevalence underscores the critical role of empirical evidence in refining our understanding and also indicates the potential dynamics of disease in the study populations. Other studies have also found shifting relative a prior information (Branscum et al., 2004). In addition, the age structure of the sampled population (approximately parity) has been shown to influence the test positivity in this study and others (Cárdenas et al., 2019; Lindahl et al., 2019). We highlight that the prevalence estimates may not be appropriate to other regions and populations, especially for young animals and other population groups other than PR and ZR regions. However, the *Se* and *Sp* estimates of the four diagnostic tests are robust and reliable, making these *Se* and *Sp* estimates potentially applicable in disease screening and surveillance efforts in commercial dairy farms in Henan and potentially other contexts.

This study has some potential dairy herd selection biases due to only six dairy herds being enrolled from two regions, so the prevalence estimates are not representative of the overall prevalence of the entire population in PR and ZR, as well as other regions of Henan Province. In addition, replacement heifers and calves were not included in this study since they were not considered to be primarily affected by *Brucella*. Additional caution is required when applying these diagnostic tests to replacement heifers and calves. For example, consideration may be given to lowering the cut-off values for continuous tests (FPA and C-ELISA) in calves and replacement heifers because they are less likely to be exposed to *Brucella* than cows.

3.5.5 Conditional dependence among tests

Our analysis revealed the presence of conditional dependence among all evaluated tests. Specifically, *Se* and *Sp* of SAT are highly dependent on the RBT results, meaning the dependence must be incorporated to calculate the combined sensitivity and specificity when performing serial or parallel testing. In our study, we observed that the combined sensitivity in the RBT-SAT series was 60.9% when accounting for the dependence, whereas it was 55.6% when the dependence was not considered. Therefore, in the presence of conditional dependence, omission of conditional dependence in the calculation of joint specificity in series will lead to underestimated *Se* and overestimated *Sp* in serial testing, while the opposite bias occurs in parallel testing. Unfortunately, this independence assumption has often been disregarded in most studies in China (Chapter 2), which can introduce bias into the results and have potentially significant consequences in prevalence estimation and risk analysis. Georgiadis et al. (2003)

suggested that when the conditional correlations are low (≤ 0.2) the conditional independence assumption can be ignored with limited influences. On the opposite, when the correlations are high (> 0.2), the conditional dependence model should be preferred. The eight correlation coefficient estimates between FPA and C-ELISA are all larger than 0.3, indicating that there is also a high dependence between FPA and C-ELISA regardless of RBT, SAT results, and disease status. However, the conditional dependence between binary tests (RBT and SAT) and continuous tests (FPA and C-ELISA) is only evident in the presence of disease, not in non-disease states (See Figure S 3.3 in Appendix 2). Hence, a semi-dependent model that only incorporated a correlation between binary tests and continuous tests given the disease status could be developed in future studies (Kostoulas et al., 2006). Our sensitivity analysis also captured the conditional dependence between FPA and C-ELISA tests, where the model was only sensitive to SAT conditional *Se* and *Sp*. This observation may arise from the use of the identical SAT conditional *Se* and *Sp* given RBT results and flat prior distributions in sensitivity analysis, however; there are currently no published reports on SAT conditional *Se* and *Sp* given RBT. This potential concern could be addressed in future empirical studies by constraining prior distribution given another test result (e.g., SAT $Se_{C[2]}$ > SAT $Se_{C[1]}$, 2 means given RBT-positive, 1 means given RBT-negative) or by using different parameters that remove implausible prior values rather than flat priors (Johnson et al., 2019).

3.6 Conclusions

We built a novel Bayesian Latent Class Model that integrates both binary and continuous testing outcomes and additional fixed and random effects to calibrate the optimal cut-off values by maximizing Youden Index. Our findings show that sensitivity estimates of each of the four tests vary between 69.7% and 89.9%, and specificity estimates range from 97.1% to 99.6%. The optimal cut-off values for FPA and C-ELISA kits are 94.2 mP and 0.403 PI, respectively. Our modeling approach is broadly applicable to determining an optimal cut-off value and estimating diagnostic performance in the absence of a gold standard test. It has the potential to enhance diagnostic accuracy and contribute to more reliable disease detection and management strategies in animal disease control programs.

3.7 Acknowledgements

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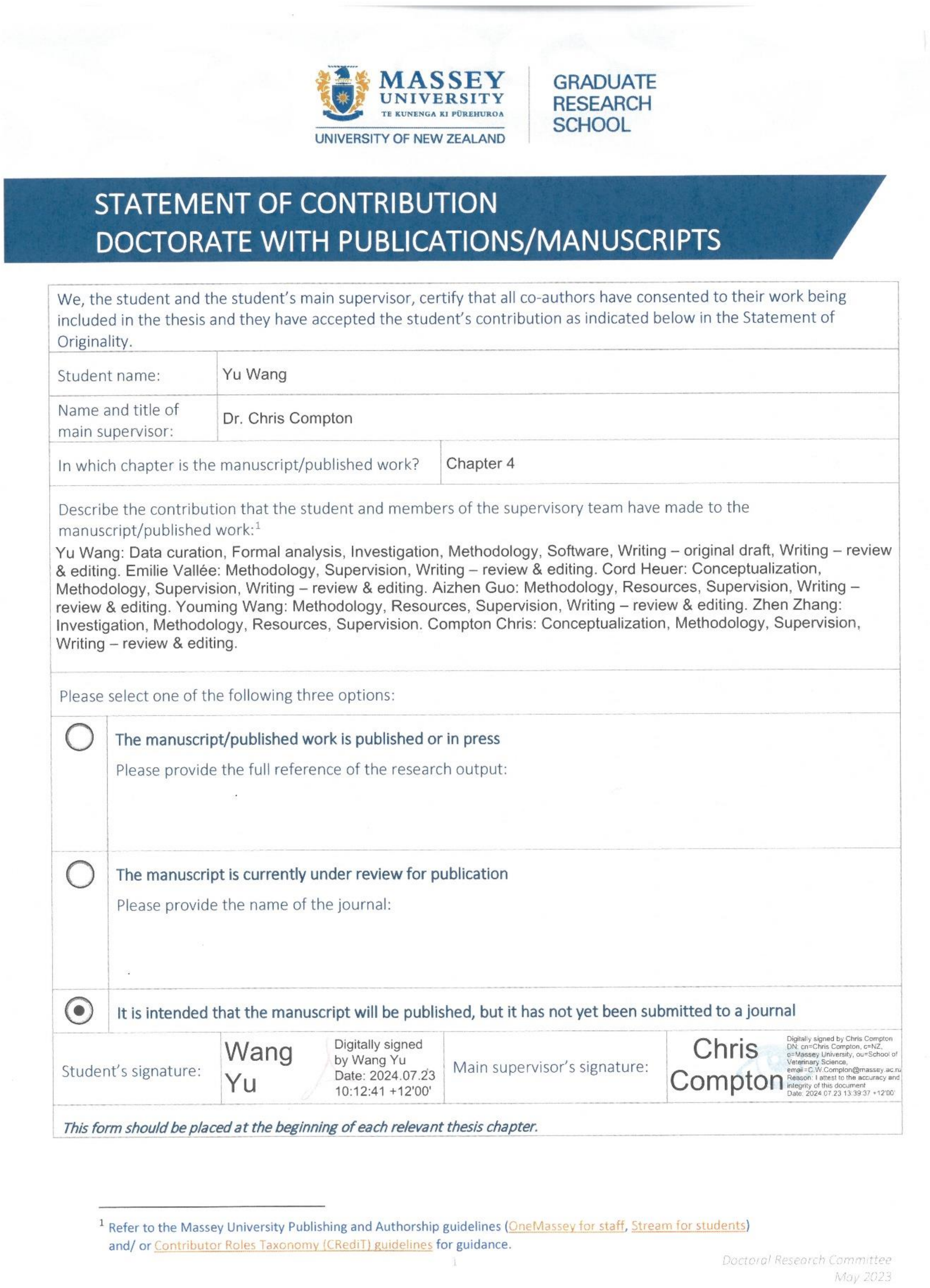

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Chapter 4 Exploring farm biosecurity practices and perspectives on *Brucella* spp. control in Henan Dairy Herds, China

Yu Wang[a], Emilie Vallée[a], Cord Heuer[a], Youming Wang[b], Aizhen Guo[c], Zhen Zhang[d], Chris Compton[a]

[a] EpiCentre, School of Veterinary Science, Massey University, Private Bag 11-222, Palmerston North, New Zealand

[b] China Animal Health and Epidemiology Center, Qingdao, China

[c] National Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China

[d] Henan Dairy Herd Improvement Center, Zhengzhou, Henan, 450045, China

Preface

In addition to relying on diagnostic tests to identify infected animals and herds, how to manage and prevent brucellosis requires adequate farm biosecurity practices. However, the adoption of farm biosecurity practices in China is heavily influenced by farmers' perceptions of disease risk and motivations. Chapter 2 has summarized key risk factors identified in Chinese dairy herds such as the introduction of new animals, shared vehicles, hygiene deficiencies, and inadequate isolation of sick animals. Applying appropriate farm biosecurity measures could effectively mitigate the within-herd and between-herd disease transmission. However, the current farm biosecurity practices and motivations regarding brucellosis control among farm stakeholders remain unknown, representing a knowledge gap for the integrated bovine brucellosis control programs in China.

Chapter 4 explores the current farm biosecurity practices and motivations toward brucellosis control among Henan dairy farms through qualitative analysis. These findings could directly provide actionable insights to inform targeted biosecurity training programs, particularly for farms with suboptimal biosecurity implementation, thereby enhancing overall disease management efforts.

4.1 Abstract

Zoonotic diseases, particularly *Brucella* spp., pose significant challenges to the Chinese dairy industry, impacting animal productivity and public health. Farm biosecurity stands as a cornerstone in dairy farming strategies aimed at infectious disease management. However, the efficacy of these measures highly depends on farmers' awareness and compliance, influenced by various factors, including demographics, disease knowledge, and economic considerations. Understanding current farm biosecurity practices and relevant determinants can inform evidence-based farm biosecurity training programs. This study aimed to investigate the adoption of biosecurity practices and dairy farmers' motivations toward *Brucella* spp. in a sample of dairy herds in Henan Province, China. A cross-sectional study was conducted through interviews using a closed questionnaire in 96 Henan dairy herds. Multiple correspondence analysis (MCA) and agglomerative hierarchical cluster analysis (HCA) were performed to explore associations between adopting biosecurity practices and demographic variables and identify potential farm clusters based on these practices. MCA revealed that the first two dimensions elucidated 39.3% of the dataset's variability. The first dimension primarily reflected factors related to animal procurement, testing, and quarantine for purchased animals, while the second dimension focused on the presence of pets within herds and their access to cattle housing areas. Two distinct clusters of dairy herds were identified, each exhibiting disparate profiles in adopting biosecurity practices. Cluster 1, distinguished by its superior adherence to biosecurity practices compared to Cluster 2, was associated with medium to large herd sizes and higher educational levels among respondents. Financial constraints emerged as the primary challenge to implementing biosecurity practices, followed by technical feasibility and practicability. Tangible economic benefits from biosecurity practices are a significant motivator for stakeholders. These findings offer evidence-based insights to inform future farm biosecurity training programs in Henan dairy herds.

Keywords: farm biosecurity, *Brucella* spp., dairy herds, multiple correspondence analysis (MCA), hierarchical cluster analysis (HCA)

4.2 Introduction

Farm biosecurity practices encompass a range of procedural measures designed to prevent disease transmission between animal groups on a farm and across farm premises, regions, and countries (Robertson, 2020). Multiple studies have demonstrated that biosecurity measures can cost-effectively prevent the introduction of infectious agents and within- and between-herds transmission and improve animal welfare and production performance (Fountain et al., 2022; Han et al., 2020; Sorin-Dupont et al., 2023). Farm biosecurity is integral to disease control and elimination programs of intensive dairy farming worldwide. Noteworthy, the implementation and effectiveness of farm biosecurity schemes depend heavily on the awareness and compliance of farmers, predominantly as a voluntary effort rather than a mandated, government-enforced provision (Aleri et al., 2020; Denis-Robichaud et al., 2019b; Ornelas-Eusebio et al., 2020). Multiple factors, such as demographics and disease knowledge, may influence the adoption of farm biosecurity practices (Denis-Robichaud et al., 2019a; Sayers et al., 2013). A comprehensive understanding of current farm biosecurity practices and potential factors influencing dairy farmers' acceptability and feasibility is essential to successfully implementing disease control and elimination programs (Dhand et al., 2021).

Bovine brucellosis, primarily caused by *Brucella abortus* (*B. abortus*), is a significant zoonotic disease affecting animal welfare and productivity (Wang et al., 2021b; Zhang et al., 2018). Despite the lessons learned from successful *B. abortus* eradication programs in some countries (Zhang et al., 2018), controlling this disease remains challenging in regions where *B. abortus* is endemic, particularly in nations with substantial animal populations, such as China and India (Holt et al., 2021; Wang et al., 2021b). Many farm practices were associated with *Brucella* spp. infection and transmission, including practices like the introduction of new animals, feeding raw milk to calves, and commingling with species other than those being primarily raised (Cárdenas et al., 2019; Musallam et al., 2015a; Wang et al., 2022). Cui et al. (2014) suggested that not having dedicated working boots and cleaning boots are potential risk factors associated with brucellosis, which may bring contaminants into the farm or cross-contaminate between cattle housing areas. These critical risk factors can be effectively managed through implementing adequate biosecurity practices to minimize the risk of transmission of *Brucella* spp. and other pathogens. Furthermore, the Chinese government document also requires accredited *Brucella*-free dairy herds to satisfy a series of biosecurity management measures (China Animal Disease Prevention and Control Center, 2023). 'Best' farm biosecurity practices are continuously evolving to mitigate risks that arise from (re-)emerging pathogens and various pathways, such as food and water supplies, and wildlife (Robertson, 2020).

Henan Province launched a five-year action plan for animal brucellosis in 2022, including measures such as vaccination, test-and-culling, accreditation of *Brucella*-free farms/zones, and enhanced farm biosecurity (Department of Agriculture and Rural Affairs of Henan Province, 2022a). Despite a consensus among farmers, researchers, and veterinary authorities regarding the pivotal role of farm biosecurity in combatting zoonotic diseases (Chen et al., 2021), the adoption of biosecurity measures remains unknown among dairy herds in Henan Province, China. Acquiring information on existing biosecurity measures and differences in implementation between herds may offer a fresh perspective to inform *Brucella* spp. control strategies in Henan dairy herds.

This study aimed to investigate (1) farm biosecurity practices and (2) opinions and perspectives regarding *Brucella* spp. control programs among Henan dairy farmers. Insights gained from the study will be essential for designing targeted interventions that align with the needs and expectations of the Henan dairy farming community.

4.3 Materials and Methods

The study was analyzed and written following the STROBE-Vet reporting guidelines (Sargeant et al., 2016).

4.3.1 Study population

A cross-sectional study was conducted through a closed questionnaire between June and October 2022 to investigate farm biosecurity practices and gather opinions about *Brucella* spp. control programs among dairy farmers in Henan Province, China. The source population comprised 150 dairy herds listed in the Henan Dairy Herd Improvement (DHI) Centre database, representing more than 90% of the dairy population in Henan province. Henan DHI is a government-funded organization that has provided free milk component monitoring and paid early pregnancy testing services to Henan dairy herds. This dairy herd group included almost all intensive dairy herds ($N \geq 100$) in Henan Province, but non-intensive dairy herds ($N < 100$) were not included in the Henan DHI database due to logistical constraints. The lack of contact with these small-scale dairy herds hindered us from accessing this part of the dairy population. Initial contact with all 150 DHI-listed farms was via text message or phone call to obtain visit permits, followed by scheduling in-person interviews with consenting participants. On the day of the farm visit, the interviewees were selected based on their familiarity with routine farming management, involvement in farm disease control, willingness to participate in the survey, and time availability. Farm managers and veterinarians were the preferred interviewees in this study.

The principal investigator (Y.W.) conducted the interviews and recorded responses using a paper questionnaire. Participants had the right to withdraw from the study or refuse to answer any questions throughout the survey as participation was voluntary.

The sample size was calculated by assuming a proportion of 30% of farms adopting biosecurity measures with a 95% confidence level, allowing for a 10% error rate using the 'epiR' package (Stevenson, 2021; Stevenson et al., 2022). The choice of 30% of dairy herds adopting biosecurity measures was assumed based on personal communication with Henan DHI farm service staff. After adjusting for a finite population size of 150 and a 60% response rate (Dohoo et al., 2009), a minimum of 89 farms was required.

4.3.2 Questionnaire design

A questionnaire, primarily comprising close-ended questions, was developed through a two-stage approach: pooling questions from currently published farm biosecurity surveys (Kristensen et al., 2011; Sayers et al., 2013) and utilizing government-recommended farm biosecurity practice guidelines (Chen et al., 2020). A pilot survey was conducted on three dairy farms independent of the study population to refine the content and readability of the questionnaire. The questionnaire was initially developed in English and then translated into Chinese for the survey. The survey was conducted in person during a farm visit. The survey was a 20-30 minute interview using the questionnaire attached as Appendix 3. The studied variables were categorized into five broad categories based on the questionnaire. Category 1 variables described the demographic information of dairy herds and respondents. Category 2 variables included questions on routine management practices related to dairy herd reproduction activities, such as mating method, inseminator source, and prior handling of milk fed to calves. Category 3 variables contained essential biosecurity practices that focused on the purchase and sale of animals and contact with visitors and vehicles. Category 4 variables investigated *Brucella* spp. testing and vaccination measures implemented on dairy herds. Category 5 variables explored the motivation and challenges perceived by the respondents related to the implementation of farm biosecurity programs. All questions in the questionnaire were targeted for the 2021-2022 production season.

4.3.3 Data analysis

Data from the questionnaire survey were initially transferred into Microsoft Excel (Microsoft Office version 365; Redmond, WA, USA) and then imported into the R statistical software version 4.1.2 (R Core Development Team, 2021) for data analysis. Descriptive statistics were used to summarize continuous (median and range) and discrete (count and proportion) variables. Farm biosecurity variables were included as active variables in the analysis, and demographic variables were set as additional categorical variables (N = 7; region, gender, age, education, job role, year of dairy farming, and herd size). Given the substantial right skew in herd size distribution, herd sizes were categorized as small (≤ 400), moderate (400 - 1000), and large (≥ 1000) to achieve balanced proportions. Since most herds had abortion cases, we dichotomized them into high (≥ 8.0 cases/100 calvings) and low (< 8.0 cases/100 calvings) abortion rates by median (8.0 cases/100 calvings). Low-frequency (<5%) categories were merged into adjacent categories to minimize their impact on the overall results. For example, respondents under 35 were aggregated into the 35-44 age group, subsequently classified as the under 45 age group.

For missing values, a distinct category (unknown) was created for each question to evaluate their potential impacts on study results.

Multiple correspondence analysis (MCA) was used to analyze the current adoption of different farm biosecurity practices in dairy herds in Henan Province, allowing visualization of potential relationships with categorical variables while reducing the complexity of correlated variables (49 biosecurity variables vs 96 respondents) to a more manageable subset for further investigation (Husson et al., 2017). The MCA dimensions were specified as two to facilitate a 2-dimensional graphical plot. These dimensions were shown in descending order of the amount of variations they can account for, and the variables that contribute the most to the dimensions were identified. The R-squared (R^2) and its v-test for a variable within the 1st and 2nd dimensions in MCA were calculated to represent the proportion of the total variability in the data set that was explained by the MCA model (Denis-Robichaud et al., 2019a; Husson et al., 2017). Dairy herds were subsequently classified into clusters based on the similarity of their biosecurity practices in MCA, using a Hierarchical Cluster Analysis (HCA) based on Ward's method (Ornelas-Eusebio et al., 2020). The number of optimal clusters was determined by automatically evaluating available indexes of the NbClust package version 3.0.1 (Charrad et al., 2014). The proportion of farms within each cluster for each category was calculated with a 95% Wilson confidence interval (95% CI) using the epiR package version 2.0.62 (Stevenson et al., 2022). Compared to the overall adoption, the over-representation of variables within each cluster was determined through a hypergeometric test (Husson et al., 2017). The hypergeometric test is appropriate for testing the over-representation of a specific biosecurity practice in each cluster because it evaluates whether a particular category (e.g., adoption of a practice) is significantly more prevalent in a subset (cluster) than expected by chance based on the entire population, without assuming independent sampling. The FactoMineR version 2.8 (Lê et al., 2008) and factoextra version 1.07 (Kassambara et al., 2020) packages were used to perform the MCA and HCA and to visualize the results.

4.3.4 Ethics approval

The study received an ethical approval from the Massey University Human Ethics Committee (Approval number: SOA 21/60). Written informed content was obtained from each participant before the study. All collected data was anonymized and kept confidential to protect the interests of the participants.

4.4 Results

4.4.1 Response rate and participant demographics

Ninety-six of 150 invited farmers participated in the study, resulting in a final response rate of 64.0%. The distribution of these herds is shown in Figure 4.1. The demographics of the 96 respondents are summarized in Table 4.1. Most respondents were male and older than 45 years of age. Educational backgrounds were mainly at primary and secondary levels, with limited achievement in higher education. Half of the respondents held positions as herd managers, followed by veterinarians actively engaged in routine herd management.

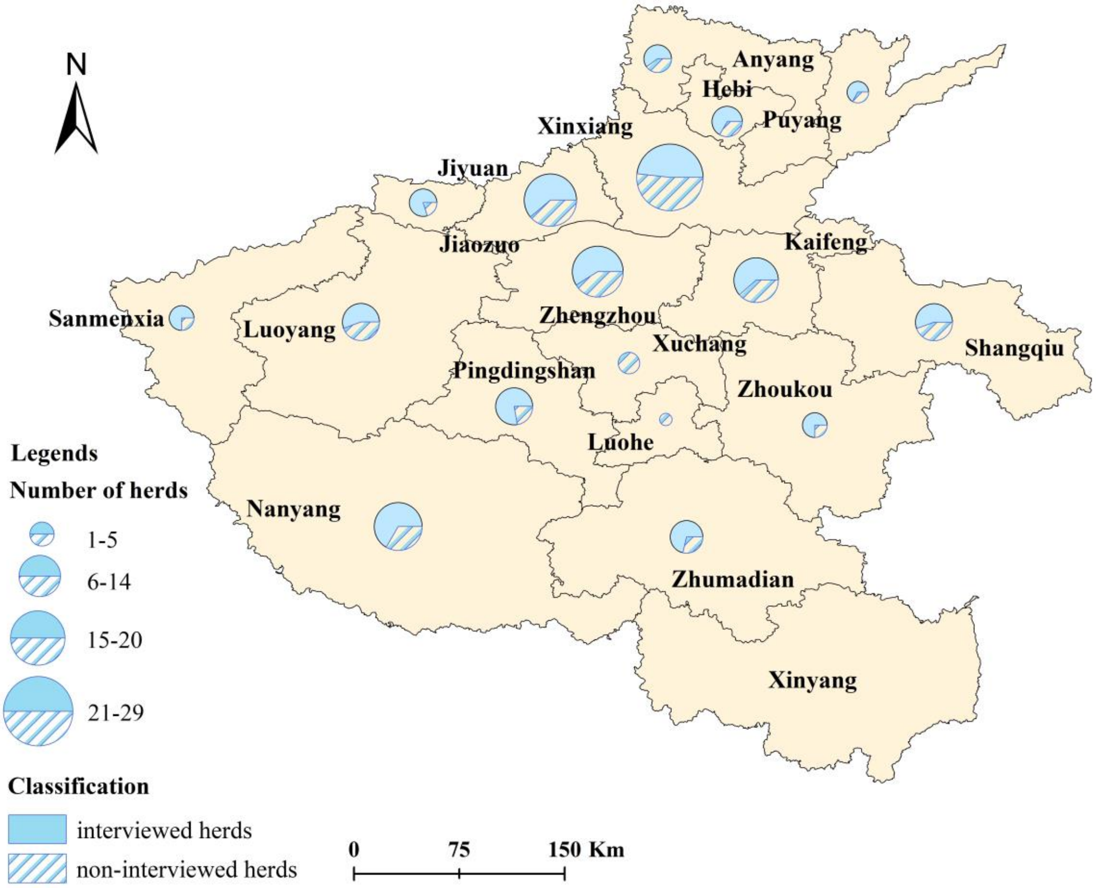

Figure 4. 1 Representative distribution of investigated dairy herds in Henan Province (Note: No dairy herds meeting the study inclusion criteria existed in Xinyang).

Table 4. 1 Descriptive results regarding respondents' profile and participating herds

Variable	Category	Number (N = 96)	Proportion (%)
Gender	Male	80	83
	Female	16	17
Age	<45	24	25
	45-54	34	35
	>54	38	40
Education	Never	6	6
	Primary school	31	33
	High school	54	56
	Higher education	5	5
Work duty	Owner	6	6
	Manager	49	51
	Veterinarian	31	32
	Others	10	11
Length of working (year)	Junior (≤9)	26	27
	Intermediate (9<N<15)	38	40
	Senior (≥15)	32	33
Herd size	Small (≤ 400)	38	40
	Moderate (400<N<1000)	34	35
	Large (≥1000)	24	25

4.4.2 Description of survey results

Artificial insemination was the predominant mating method (95/96), with 39 herds sharing inseminators with other herds (Table 4.2). Only 15% of herds practiced boiling or pasteurizing raw milk before feeding it to calves. In the 2021-2022 calving season, most herds reported at least one abortion case occurring in their cows. The average and median abortion rate was 7.2 and 8.0 cases per 100 calvings, respectively. The popular handling practices for aborted products were through burning or burial. Approximately half of the herds treated aborted cows similarly to those undergoing regular calving, followed by isolation and antibiotic treatment (23%) (Table 4.2).

Introduction of animals occurred in 49% of the herds, with replacement heifers making up the majority (60%) (Table 4.3). Only a few dairy herds implemented testing (15%) and quarantine measures (11%) for purchased animals. Inquiries about the *Brucella* infection status or vaccination history of purchased animals were rare (4/47). About 17% of respondents expressed a moderate or substantial likelihood of making such inquiries when purchasing animals (Figure 4.2). Thirty-eight percent of herds shared equipment or vehicles with other dairy herds (Table 4.4). While 60% of herds maintained records of visitors and vehicles, 15% allowed visitors access to cattle housing sheds. The personnel sanitation channel is a disinfection facility installed at the entrance of the farm, operated by farm workers when entering and leaving. Once opened, visitors and workers will be disinfected with a disinfectant spray, effectively reducing the contaminants on the surface of visitors and workers. A quarter of herds owned personnel sanitation channels, and 56% of respondents on herds with available sanitary facilities expressed a moderate or substantial likelihood of using it before entering production areas (Figure 4.2). Disinfection pools at vehicle entrances were available in half of herds, with three-quarters replacing disinfectant water at least weekly. A quarter of respondents raised dogs on their farms, and 28% reported entering cattle housing areas. Rodents (e.g., mice and weasels) were commonly found within herds, with 63% of herds implementing regular rodent control plans.

Most dairy herds (73%) conducted laboratory tests for *Brucella* spp. in 2021-2022 (Table 4.5). Vaccination was the primary reason (85%) for herds discarding testing. Half of the respondents chose not to disclose previous test results. Sixteen percent of herds had tested positive at least once, while 34% declared no positive test results. In case of a positive test result, a quarter of dairy herds conducted follow-up tests on the remaining animals that had not been included in the previous round of testing, and 40% reported suspicious cases to veterinary departments. Additionally, 63% of herds have used vaccines against *Brucella* spp. in the past three years, primarily utilizing the *B. abortus* A19 strain. Vaccination strategies varied, with 55% employing a single calf vaccination and 32% opting for calf vaccination combined with a booster injection before the first mating.

Table 4. 2 Frequencies and proportions of various general practices related to reproductive activity

Questions	Options	Total of response	Number of answers	Frequency (95% confidence interval, %)
What is your mating method?	Artificial insemination	96	95	99 (94 – 100)
	Natural mating		1	1 (0 – 6)
Do you have an exclusive breeder/inseminator?	Yes	96	57	59 (49 – 69)
What is your priority destination for culled cattle?	Sale to slaughterhouse	96	13	14 (8 – 22)
	Sale to cattle traders		72	75 (65 – 83)
	Sale to other farms		11	11 (7 – 19)
How often do you disinfect the calving shed per month?	Frequency ≥ one time weekly	96	50	52 (42 – 62)
	Frequency < one time weekly		46	48 (38 – 58)
Do you process the raw milk before feeding calves? (e.g., boiling or pasteurization)	Yes	96	14	15 (9 – 23)
What kind of calving system does your farm use?	Year-round calving	96	84	88 (79 – 93)
	Seasonal calving		12	12 (7 – 21)
Where does semen come from?	Commercial company	96	91	95 (88 – 98)
	Imported semen		1	1 (0 – 6)
	Unknown		4	4 (2 – 10)
Did you observe any cows that aborted or had a stillborn calf in 2021-2022?	Yes	96	89	93 (86 – 96)
Did your herd have a high abortion rate (≥8.0 cases/100 calvings) in 2021-2022?	Yes	91	48	53 (43 – 63)
If YES, what did you do with the aborted products?	Burn or bury it	89	78	88 (79 – 93)
	Feed it to other animals (e.g., dogs)		11	12 (7 – 21)
How to deal with aborted cows on your farm?	Manage like normally calving cows	96	46	48 (38 – 58)

	Isolation and treatment as sick cows	20	21 (14 – 30)
	Consult a veterinarian for advice	7	7 (4 – 14)
	Conduct laboratory test and then make a decision based on test results	16	17 (11 – 25)
	Premature culling	7	7 (4 – 14)

Table 4. 3 Frequencies and proportions of various biosecurity practices related to cattle trade

Questions	Options	Total of response	Number of anwser	Frequency (95% confidence interval, %)
Have you purchased cattle in 2021-2022?	Yes	96	47	49 (39 – 59)
If YES, which age group did you usually purchase?	Young heifers	47	9	19 (10 – 33)
	Replacement heifers		28	60 (45 – 72)
	Adult cows		10	21 (12 – 35)
Have you tested for *Brucella* in purchased animals?	Yes	47	7	15 (7 – 28)
Where did you purchase cattle from?	Neighbor farms	47	23	49 (35 – 63)
	Cattle traders		14	30 (19 – 44)
	Suppliers from other provinces		10	21 (12 – 35)
Have you quarantined the purchased cattle?	Yes	47	5	11 (5 – 23)
What is the main factor affecting the isolation practice?	Cost	47	3	6 (2 – 17)
	Lack of isolation shed		16	34 (22 – 48)
	Increased workload		13	28 (17 – 42)
	Unknown role of isolation		15	32 (20 – 46)
Did you ask about the brucellosis status of purchased animals?	Yes	47	4	9 (3 – 20)
Did you ask about the vaccination history of purchased animals?	Yes	47	4	9 (3 – 20)
Did you sell cattle in 2021-2022?	Yes	96	96	100 (96 – 100)
Where were the buyers from?	Neighbor farms	96	30	31 (23 – 41)
	Cattle traders		50	52 (42 – 62)
	Abattoir		16	17 (11 – 25)

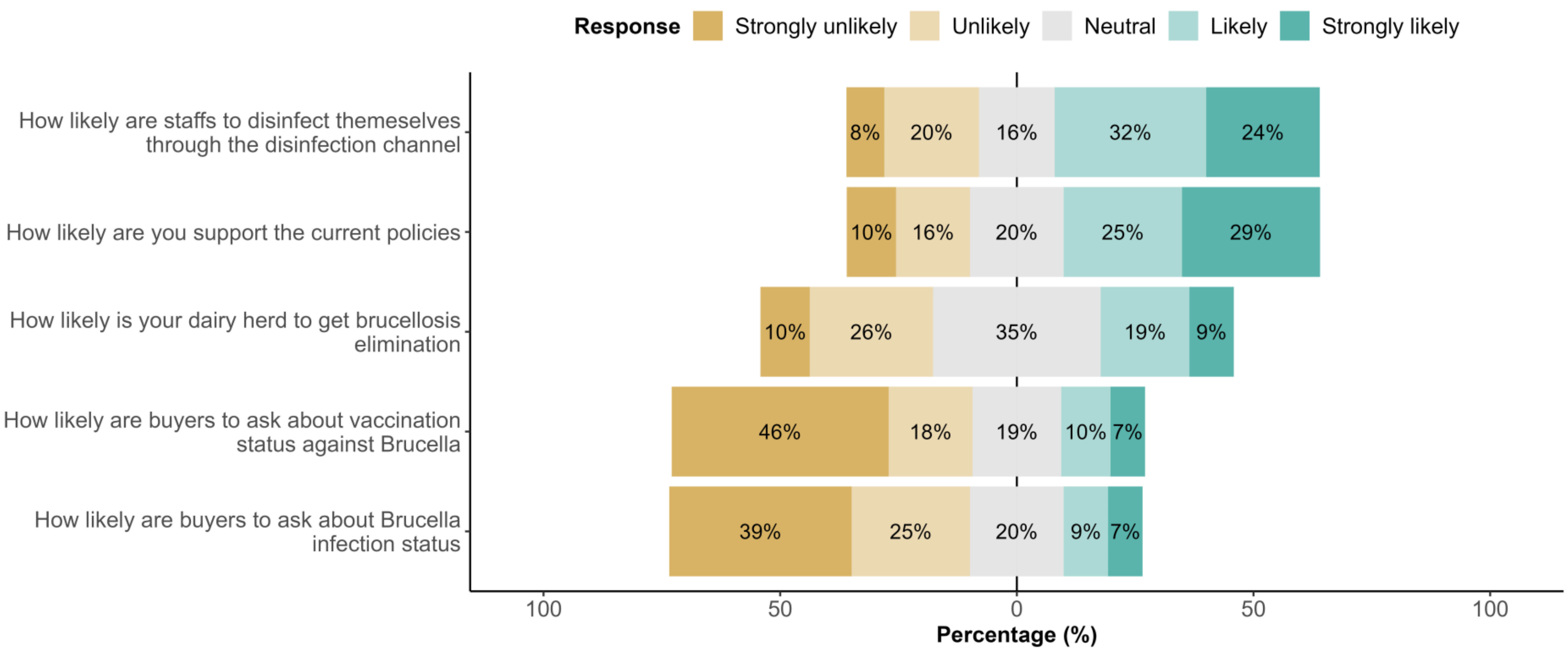

Figure 4. 2 Preferences and opinions of respondents from Henan dairy herds on *Brucella* spp. biosecurity practices. The percentages on each cell of each row represent the likelihood of response to related questions.

Table 4. 4 Frequencies and proportions of various biosecurity practices related to contact and hygiene of visitors and vehicles, wildlife

Questions	Options	Total of response	Number of answers	Frequency (95% confidence interval, %)
Were there cattle from other farms that are raised on your farm?	Yes	96	2	2 (1 – 7)
Do you know the status of the brucellosis on the neighbor farm?	Yes	96	17	18 (11 – 27)
Do you know the vaccination against brucellosis on the neighbor farm?	Yes	96	30	31 (23 – 41)
Do you have shared facilities with other farms? e.g., milk tankers	Yes	96	36	38 (28 – 47)
Do you have records of visitors and vehicles?	Yes	96	60	63 (53 – 72)
Are visitors and vehicles allowed to access cattle housing sheds?	Yes	96	11	12 (7 – 19)
Do farm workers wear masks and gloves while working?	Yes	96	49	51 (41 – 61)
Do farm workers clean and disinfect boots and clothes?	Yes	96	61	64 (54 – 72)
Is there a disinfection channel at the entrance of the farm?	Yes	96	25	26 (18 – 36)
Is there a vehicle disinfection pool at the entrance of the farm?	Yes	96	46	48 (38 – 58)
If YES, is the disinfectant solution changing frequency at least once a week on your farm?	Yes	46	34	74 (60 – 84)
Please indicate the domestic animals that exist on your farm	None	96	57	59 (49 – 69)
	Cat		15	16 (10 – 24)
	Dog		24	25 (17 – 35)
If YES, have these animals entered cattle sheds?	Yes	39	11	28 (17 – 44)
Do you see rodents on your farm? (e.g., mice and weasels)	Yes	96	89	93 (86 – 96)
Do you have a regular rodent control plan on your farm?	Yes	96	60	63 (53 – 72)

Table 4. 5 Testing and vaccination practices among Henan dairy herds

Questions	Options	Total of response	Number of answers	Frequency (95% confidence interval, %)
Have you had your cattle tested for brucellosis in 2021-2022?	Yes	96	70	73 (63 – 81)
What was the main reason for not testing?	Cost	26	4	15 (6 – 34)
	Have used vaccine		22	85 (66 – 94)
How often were your animals tested for brucellosis?	Annual	70	11	16 (9 – 26)
	Half-year		37	53 (41 – 64)
	Seasonal		15	21 (13 – 32)
	Occasional		7	10 (5 – 19)
What kind of animals were tested?	Aborted animals	70	24	34 (24 – 46)
	All cattle		2	3 (1 – 10)
	Random sampling		44	63 (51 – 73)
Have you tested positive against brucellosis at least one animal in your herd in 2021-2022?	Yes (Known positive)	96	15	16 (10 – 24)
	No (Known negative)		33	34 (26 – 44)
	Unknown (Missing)		48	50 (40 – 60)
If YES, will you do follow-up testing for other animals?	Yes	15	4	27 (11 - 52)
What was usually done with test-positive animals?	Sent to abattoir	15	2	13 (4 – 38)
	Sale to traders		5	33 (15 – 58)
	Keed feeding		1	7 (1 – 30)
	Isolation and treatment		1	7 (1 – 30)
	Report it to veterinary department		6	40 (20 – 64)
Have you ever used vaccines against	Yes	96	60	62 (53 – 72)

Questions	Options	Total of response	Number of answers	Frequency (95% confidence interval, %)
brucellosis on your farm?				
What was your main reason for not vaccinating?	Adaptation of other measures	35	20	57 (41 – 72)
	Poor vaccine efficacy		5	14 (6 – 29)
	Claim disease free		4	11 (5 – 26)
	Policy regulation		3	9 (3 – 22)
	Interference with tests		3	9 (3 – 22)
	Cost		1	3 (1 – 15)
Which vaccines did you use?	*Brucella abortus* A19 strain	60	26	43 (32 – 56)
	Brucella abortus A19-ΔVirB12 strain		22	37 (26 – 49)
	Brucella suis S2 strain		12	20 (12 – 32)
Which of the following vaccination procedures did you use?	Once at six months of age	60	33	55 (42 – 67)
	Once at six months of age and a booster before the first mating		19	32 (21 – 44)
	Once at six months of age and an annual booster before mating		8	13 (7 – 24)

4.4.3 Motivations and challenges towards *Brucella* spp.

The majority of respondents (62%) were familiar with the current animal brucellosis control policy in Henan province (Table 4.6). The Ministry of Agriculture and Rural Affairs (MARA) was selected as the preferred policy maker, followed by individual farmers. Only 28% of respondents expressed moderate or substantial confidence in achieving brucellosis elimination in their herds (Figure 4.2). Nevertheless, over half of respondents were likely or very likely to support the current Henan *Brucella* spp. control measures. Purchasing *Brucella*-free or vaccinated animals emerged as the most popular voluntary measure. There were considerable variations in the selection of mandatory measures (Table 4.6), with compulsory vaccination of confirmed positive dairy herds (98%) being widely accepted while test-and-culling was favored by a minority (12%). Respondents highlighted substantial financial cost (95%) and technical feasibility and practicality (79%) as the most significant challenges. The primary benefit of eliminating brucellosis was reduced risk of human infection (89%), whereas milk quality and price improvements were less recognized (41%). Respondents recommended increased compensation for culling test-positive animals to market value, scientific guidance in building farm biosecurity systems, and solid evidence of monetary benefits to incentivize compliance with current policies.

Table 4. 6 Awareness and opinions toward *Brucella* spp. control policies among Henan dairy farmers.

Questions	Options	Total of response	Number of answers	Frequency (95% confidence interval, %)
Do you observe anyone with similar symptoms on your farm? (e.g., prolonged fever and fatigue)	Yes	96	2	2 (1 – 7)
Do you know the current Henan *Brucella* control policy?	Yes	96	59	61 (51 – 71)
Who do you think should formulate brucellosis control policies?	Ministry of Agriculture and Affairs	96	60	62 (53 – 72)
	Farmers		24	25 (17 – 35)
	Dairy Industry Association		12	12 (7 – 21)
Which of the following measures would you consider voluntarily implementing on your farm? #	Purchasing *Brucella*-free or vaccinated animals only	96	85	89 (81 – 93)
	Quarantining animals brought into your farm		17	18 (11 – 27)
	Sanitary measures		40	42 (32 – 52)
	Testing off-site and on-site animals		74	77 (68 – 84)
	Testing all replacement heifers before mating		51	53 (43 – 63)
	Vaccinating at-risk groups against *Brucella*		81	84 (76 – 90)
Which of the following measures do you think should be mandatory? #	Screening annually to establish *Brucella* spp. status of each herd	96	73	76 (67 – 83)

Questions	Options	Total of response	Number of answers	Frequency (95% confidence interval, %)
	Requiring herds to declare *Brucella* spp. status at the time of sale of cattle		35	36 (28 – 46)
	Restricting animal movements for test-positive farms		25	26 (18 – 36)
	Adopting *Brucella* test-and-culling with compensation on test-positive farms		11	11 (7 – 19)
	Vaccinating positive farms		94	98 (93 – 99)
What are the most significant challenges to achieving *Brucella* spp. elimination from your farm? #	High cost input	96	91	95 (88 – 98)
	Time cost		44	46 (36 – 56)
	Insufficient replacement heifers to maintain herd structure		67	70 (60 – 78)
	Technical feasibility and practicability		76	79 (70 – 86)
What are the most significant benefits to achieving *Brucella* spp. elimination from your farm? #	Reduce the risk of human infection	96	85	89 (81 – 93)
	Improve herd productivity		46	48 (38 – 58)
	Save disease control cost		58	60 (50 – 70)
	Avoid premature culling of cows		62	65 (55 – 73)
	Raise the milk quality and price		39	41 (31 – 51)

#: multiple-choice question

4.4.4 Multivariable analysis

MCA identified a picture of the overall farm biosecurity practices; however, only the first two dimensions were explored for clarity, and 39.3% of the variance was explained. The first dimension, accounting for a substantial proportion of the variance (31.0%), primarily focused on quarantine for purchased animals ($R^2 = 0.809$) and testing for purchased animals ($R^2 = 0.807$) being the primary contributors (all *p*-values < 0.001). The second dimension, explaining 8.3% of the total variance, was characterized by the presence of pets ($R^2 = 0.516$) and pet access to cattle housing areas ($R^2 = 0.507$). The joint distribution of investigated farm biosecurity variables in dimensions 1 and 2 and their contribution values are shown in Figure 4.3.

HCA identified two distinct clusters (Figure 4.4), supported by most indexes employing a free voting method (9/30) (Charrad et al., 2014). Farm biosecurity practices that were most significantly associated with the cluster partition (Chi-squared test $p < 0.001$) in descending order included: (1) animal procurement; (2) test for purchased animals; (3) quarantine for purchased animals; (4) source of inseminator; (5) facilities sharing. Cluster 1 demonstrated superior adoption of adequate biosecurity practices (Table 4.7), mostly with middle and large herd sizes (45/47, 95.7%) and respondents with higher education levels. In contrast, Cluster 2 showed preferences for purchasing cattle without prior testing (79.6%), quarantine (83.7%), and sharing facilities with other farms (71.4%). Cluster 1 adopted rodent control practices more (91.5%) than Cluster 2 (34.7%). In contrast, the presence of pets (55.1%) and pet access to cattle housing areas (26.5%) were more frequent in Cluster 2 than in Cluster 1 (25.5% and 2.1%, respectively). More herds (24.5%) with at least one test-positive animal were found in Cluster

2, while Cluster 1 had a higher proportion of feeding calves with boiled or pasteurized milk. Notably, herds with high abortion frequency were clustered more in Cluster 2, but proper disposal of aborted cows was more frequently observed in Cluster 1.

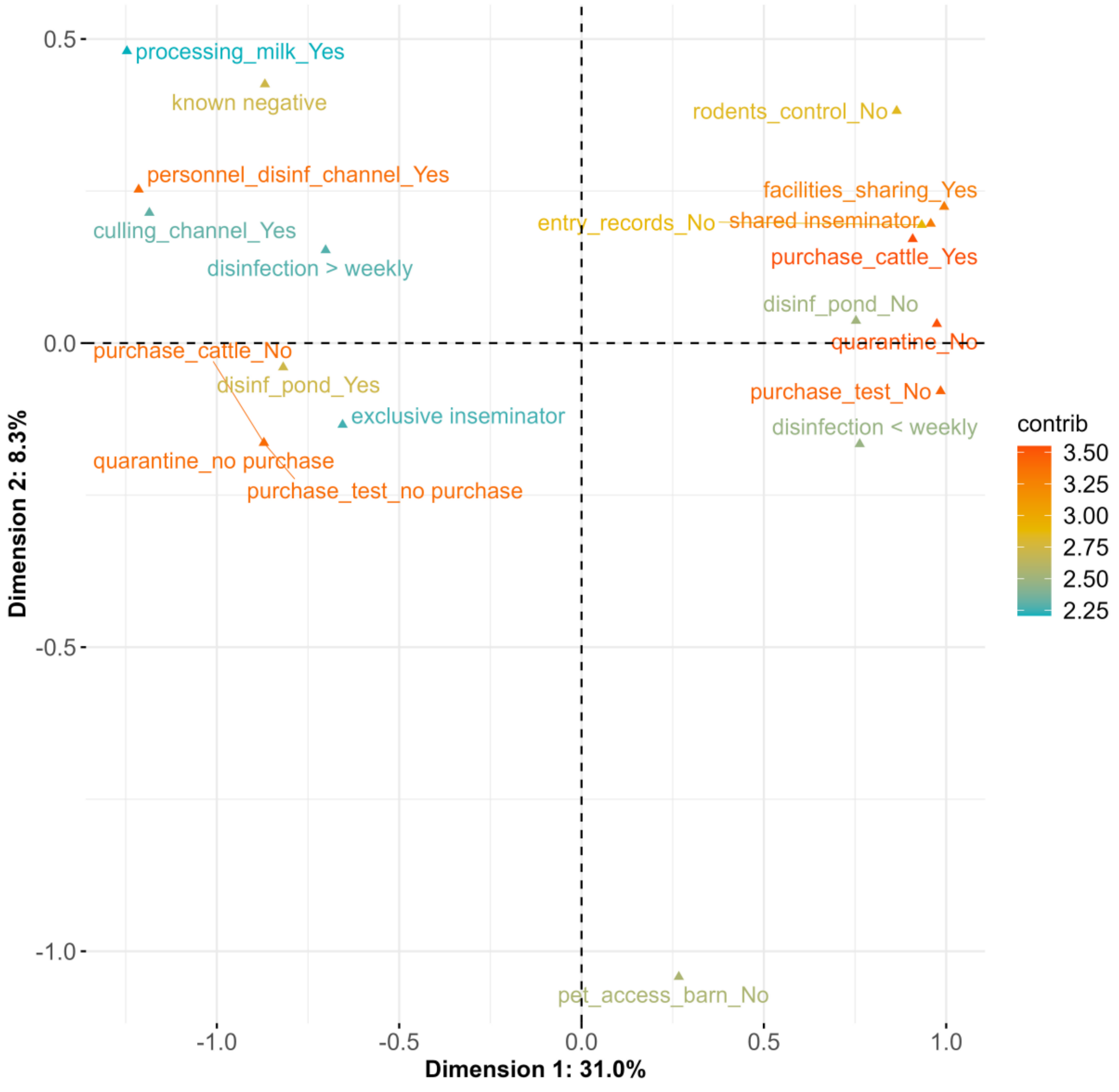

Figure 4. 3 Graphical representation of the correlations among 15 categories that contribute the most to each dimension of the 2 principal dimensions of the multiple correspondence analysis of biosecurity practices adopted on Henan dairy herds, China.

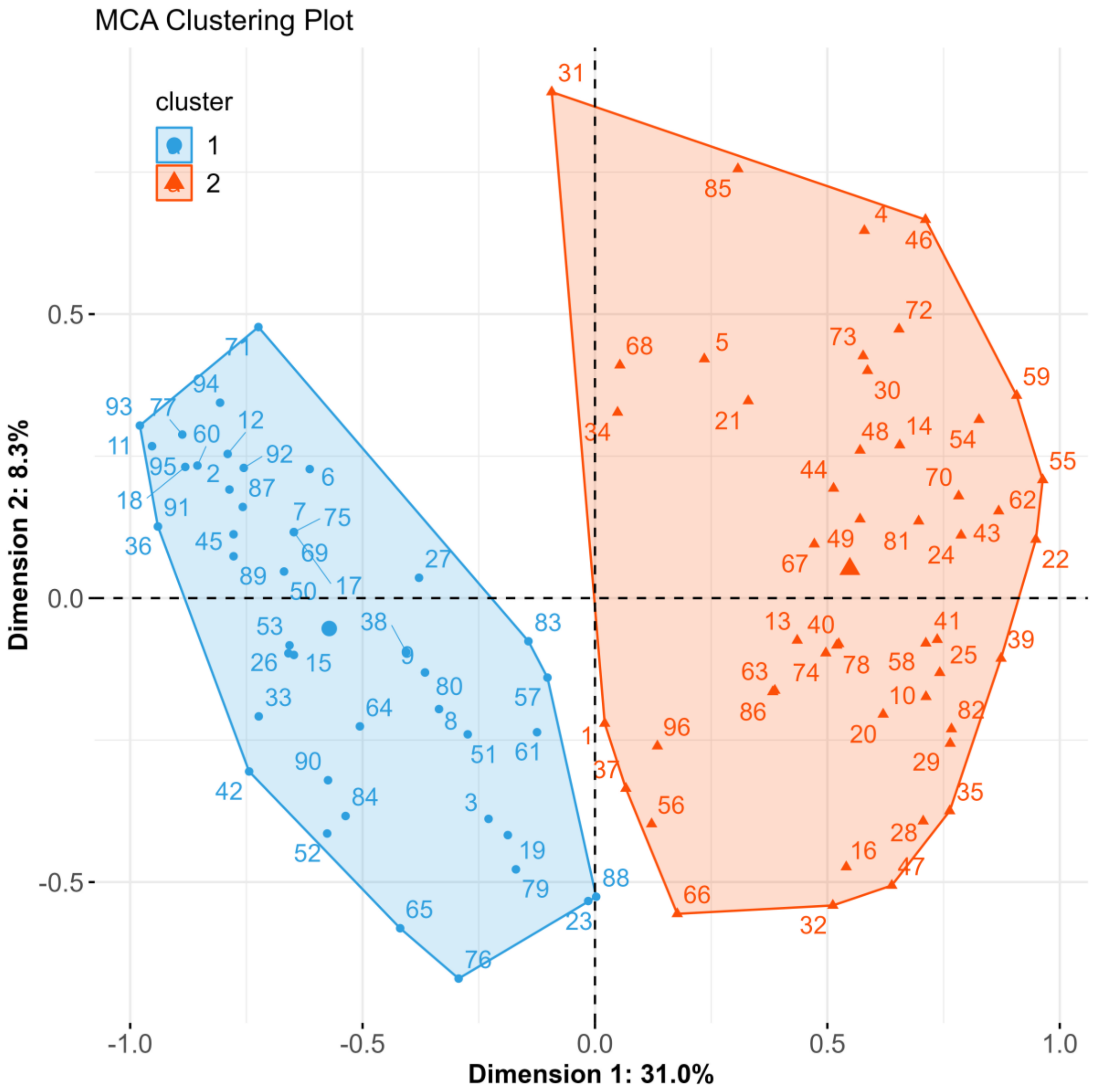

Figure 4. 4 Projection of the 96 Henan dairy herds included in this study within the two distinct clusters through the hierarchical cluster analysis and plotted in the first dimensions of the Euclidean space. Cluster 1 shows a superior adoption of adequate biosecurity practices

Table 4. 7 Frequency of farm characteristics and biosecurity practices classified by cluster obtained from the multivariate analysis on 96 dairy herds in Henan Province, China. A hypergeometric test determines the statistical difference in each category between separate cluster and the overall. No data is given when the variable is not significantly different from the overall.

Variables	Category	Cluster 1, n = 47 (%)[a]		Cluster 2, n = 49 (%)[a]		Overall, n = 96 (%)
Farm and respondent characteristics						
Herd size	Small	2 (4.3)	***	36 (73.5)	***	38 (39.6)
	Medium	24 (51.1)	***	10 (20.4)	***	34 (35.4)
	Large	21 (44.6)	***	3 (6.1)	***	24 (25.0)
Education of respondent	Never	0 (0.0)	*	6 (12.2)	***	6 (6.3)
	Primary school	13 (27.7)	0.232	18 (36.7)	0.121	31 (32.3)
	Secondary school	30 (63.8)	*	24 (49.0)	0.104	54 (56.2)
	Higher education	4 (8.5)	*	1 (2.0)	0.168	5 (5.2)
Role of the respondent within a dairy herd	Owner	0 (0.0)	*	6 (12.2)	***	6 (6.3)
	Manager	32 (68.1)	***	17 (34.7)	***	49 (51.0)
	Veterinarian	11 (23.4)	0.054	20 (40.8)	*	31 (32.3)
	Others	4 (8.5)	0.397	6 (12.2)	0.176	10 (10.4)
Farm biosecurity practices						
Inseminator source	Shared	2 (4.3)	***	37 (75.5)	***	39 (40.6)
	Exclusive	45 (95.7)	***	12 (24.5)	***	57 (59.4)
Disinfection frequency for calving facility	< 1 per week	8 (17.0)	***	38 (77.6)	***	46 (47.9)
	≥ 1 per week	49 (83.0)	***	11 (22.4)	***	50 (52.1)
Purchase cattle in 2021-2022	Yes	1 (2.1)	***	46 (93.9)	***	47 (49.0)
Test for purchased animals	Yes	0 (0.0)	**	7 (14.3)	***	7 (7.3)
	No	1 (2.1)	***	39 (79.6)	***	40 (41.7)

Variables	Category	Cluster 1, n = 47 (%)[a]		Cluster 2, n = 49 (%)[a]		Overall, n = 96 (%)
	No purchase	46 (97.9)	***	3 (6.1)	***	49 (51.0)
Quarantine for purchased animals	Yes	0 (0.0)	*	5 (10.2)	***	5 (5.2)
	No	1 (2.1)	***	41 (83.7)	***	42 (43.8)
	No purchase	46 (97.9)	***	3 (6.1)	***	49 (51.0)
Facilities sharing	Yes	1 (2.1)	***	35 (71.4)	***	36 (37.5)
Entry records for visitors and vehicles	Yes	44 (93.6)	***	16 (32.7)	***	60 (62.5)
Visitor access to the cattle housing areas	Yes	1 (2.1)	**	10 (20.4)	***	11 (11.5)
Personal protective equipment worn	Yes	34 (72.3)	***	15 (30.6)	***	49 (51.0)
Wash and disinfect clothes	Yes	42 (89.4)	***	19 (38.8)	***	61 (63.5)
Personnel disinfection channel	Yes	25 (53.2)	***	0 (0.0)	***	25 (26.0)
Disinfection pond for vehicles	Yes	39 (83.0)	***	7 (14.3)	***	46 (47.9)
Presence of pets	Yes	12 (25.5)	**	27 (55.1)	***	39 (40.6)
Pet access to cattle housing areas	Yes	1 (2.1)	***	13 (26.5)	***	14 (14.6)
Presence of rodent	Yes	46 (97.9)	**	43 (87.8)	0.062	89 (92.7)
Rodent control program	Yes	43 (91.5)	***	17 (34.7)	***	60 (62.5)
Culling channel[b]	Yes	18 (38.3)	***	0 (0.0)	***	18 (18.8)
Test against *Brucella* spp.	Yes	38 (80.9)	*	32 (65.3)	0.068	70 (72.9)
Herds with at least one test-positive in 2021-2022	Yes	3 (6.4)	*	12 (24.5)	**	15 (15.6)
	No	27 (57.4)	***	6 (12.2)	***	34 (35.4)
	Unknown	17 (36.2)	**	31 (63.3)	**	48 (50.0)
Boiling/pasteurizing milk before feeding calves	Yes	13 (27.7)	***	1 (2.0)	***	14 (14.6)
Abortion rate > median 8 cases per 100 calvings	Yes	9 (19.1)	***	39 (79.6)	***	48 (50.0)
Proper disposal of aborted cows	Yes	38 (80.9)	***	12 (24.5)	***	50 (52.1)

[a]: Statistical significance is represented according to *p*-values (* *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001); [b]: culling channel means a loading pathway for transport to sale and slaughter.

4.5 Discussion

Farm biosecurity profiles have been recently explored in Chinese poultry and pig farms (Cui et al., 2019; Li et al., 2023), but remain limited in Chinese dairy herds (Zhu et al., 2023a), particularly in the context of *Brucella* spp. This study aimed to assess farm biosecurity practices among Henan dairy herds and investigate opinions regarding current *Brucella* spp. policies among Henan dairy farmers, which provided valuable and novel biosecurity information to fill current knowledge gaps in China. The findings highlighted a considerable variation in farm biosecurity practice adoption and identified two distinct clusters of dairy herd profiles.

Cluster 1, marked by superior biosecurity practice adoption compared to Cluster 2, comprised mainly medium to large-sized herds managed by respondents who served as farm managers with higher educational levels (Table 4.7). This suggests that herd size, role within a herd, and education may significantly influence the adoption of biosecurity measures (Aleri et al., 2020; Sarrazin et al., 2014; Sayers et al., 2013). Additionally, Shortall et al. (2016) found that there may be discrepancies between farmers and veterinarians in their approach to biosecurity. Collaborative decision-making processes between farmers and veterinarians may enhance biosecurity efforts. The observed demographic differences between clusters could inform tailored biosecurity training programs, particularly for Cluster 2, to improve overall compliance within Henan dairy herds.

Artificial insemination could be a potential risk factor for *Brucella* spp. infection in cattle (Cárdenas et al., 2019; Lindahl et al., 2019). Although most Henan dairy herds (94.8%) used artificial insemination with commercial semen from certified disease-free bulls, the practice of sharing inseminators and facilities may pose contamination risks, introducing *Brucella* spp. or other pathogens between and within herds (Ana Cláudia et al., 2015; Cheng et al., 2022). Furthermore, inadequate hygiene measures for visitors, staff, and vehicles may exacerbate these risks (Megahed et al., 2022; Musallam et al., 2015a). Quarantine and hygiene measures for introduced/sick animals effectively mitigate the spread of *Brucella* spp. or other infectious diseases (Li et al., 2021). However, these mitigation measures were rarely adopted among Henan dairy herds, as identified in this study. Raising awareness among farmers to identify risks and adopt mitigation measures can help reduce the risks of disease introduction through exotic fomites.

Introduction of animals stands as an essential risk factor for *Brucella* spp. infection in China and other countries (Li et al., 2021; Musallam et al., 2015a; Wang et al., 2022). However, less than 15% of herds, particularly in Cluster 2, implemented quarantine or testing for purchased animals, similar to findings reported in Belgian cattle farms (17%) (Sarrazin et al., 2014) and Ireland dairy farms (14.5%) (Sayers et al., 2013). Challenges such as limited perception of quarantine importance and lack of quarantine facilities may hinder the adoption of quarantine (Denis-Robichaud et al., 2019b; Sarrazin et al., 2014; Zhu et al., 2023a; Zhu et al., 2023b). A closed farm management may be a preferred farming system that is self-sufficient and does not rely on outside inputs (such as semen and feed), but maintaining a closed system may not be feasible for most dairy herds due to the need for genetically superior breeds throught animal procurement. Sayers et al. (2013) recommended that the three cornerstones of farm biosecurity (including closed herd, quarantine, and testing for purchased animals) should be implemented to reduce the risk of invasion of exotic pathogens. A quarantine period of 21-28 days allows latently infected animals to show visible clinical signs (Barrington et al., 2002; McCarthy et al., 2021), but it may be longer for chronic diseases. Logistical challenges of a 21-28 days quarantine management and intangible benefits from quarantine may discourage farmers from adopting this biosecurity practice. Therefore, additional economic evidence and incentives are necessary to promote adherence to strict quarantine measures. In a setting where brucellosis is endemic like Henan, the focus of brucellosis control may shift from brucellosis eradication to mitigation and management within the herd. Vaccination could become a priority to reduce transmission and protect susceptible cattle, but farm biosecurity remains crucial as vaccination alone cannot entirely prevent the introduction of new infections from external sources, such as wildlife reservoris or purchased animals.

The presence of pets and their access to cattle housing areas posed a potential risk in Cluster 2. Recent studies have confirmed the role of these pets in *B. abortus* transmission (Wareth et al., 2017) and other infectious diseases (e.g., rabies) (Chao et al., 2021) due to their biological role as hosts to *B. abortus* and other *Brucella* species (Alamian et al., 2020; Liu et al., 2020b; Wareth et al., 2017). Dogs and cats may contract *B. abortus* through eating contaminated milk or aborted products and then shed *B. abortus* into the environment, causing environmental contamination and spillover infections for naïve animal groups and humans. Moreover, our study also indicated the role of wild animals, particularly rodents, in a comprehensive farm biosecurity system. Lovera et al. (2017) reported the prevalence of *Brucella spp.* and other pathogens infection in murine rodents in intensive dairy cattle and swine production systems, suggesting that rodents are natural reservoirs of *Brucella* spp. that maintain the persistence of the pathogen in natural hosts. Furthermore, *B. abortus* biovar 1 was isolated from Himalayan marmots in Xinjiang and Qinghai Province, China (Xue et al., 2023; Yang et al., 2022c), but there are currently no studies on *Brucella* infection in rodents in Henan Province. Evidence from an experimental test suggested marmots could come into close contact with livestock in grazing areas (Poudel et al., 2016). From a biosecurity perspective, species other than being primarily raised, including domestic and wild animals, should be prohibited from being kept and contacted within a farming property.

Feeding raw milk to calves without pasteurization or boiling could facilitate the persistence of *Brucella* spp. or other pathogens in dairy herds (Holt et al., 2011; Musallam et al., 2019). Only a minority of investigated herds (15%) boiled or pasteurized milk before feeding it to calves, with Cluster 1 (27.7%) showing higher adoption. Dairy farmers may feed calves with milk from cows that were suffering from mastitis or under therapeutic sessions to reduce milk waste and economic ‘losses’. This practice could directly cause calves to be exposed to various foodborne pathogens (e.g., *Brucella* spp.) if they are present within herds. Furthermore, cows infected with *Brucella* spp. likely shed large amounts of *Brucella* spp. in milk (Capparelli et al., 2009), which serves as the primary infection source for calves. Pasteurization or boiling of raw milk before feeding it to calves is a recognized protective factor against *Brucella* spp. and other foodborne pathogens (Tukana et al., 2017). Challenges like the availability of pasteurization facilities increased cost and workload, and awareness gaps impose difficulties in implementing milk processing practices in China and other countries (Álvarez et al., 2011).

The clustering analysis revealed a significant difference in herd *Brucella* spp. positivity, with Cluster 2 showing a higher proportion. This evidence suggested that herd prevalence assessment and disease control measures in Cluster 2 might be prioritized. However, the limited awareness of herd brucellosis status among respondents (48/96) complicates our epidemiological understanding and raises concerns about adopting the current disease control strategy. Plausible explanations for this gap may include issues such as memory lapses, social desirability biases, and technical challenges in differentiating antibodies arising from natural infection versus vaccine-induced responses (Wang et al., 2024b). Nevertheless, herds in Cluster 2 should be prioritized in subsequent control programs to yield more efficient outcomes. The inferior biosecurity practice profiles in Cluster 2 may imply a positive association with known positive herd status (Cui et al., 2019; Greening et al., 2020), and could be used for designing specific interventions.

Multiple factors may contribute to the high proportion of veterinarians (20/49) in Cluster 2. In rural areas of China, many veterinarians had limited formal education in veterinary science, relying instead on empirical, experience-based training with minimal emphasis on modern biosecurity principles (Zeng et al., 2019a). Unlike farm owners or managers, veterinarians in China often act as independent service providers rather than integrated farm employees. Consequently, their primary role is to treat individual sick animals rather than to implement herd-level disease prevention strategies. Moreover, veterinary practices in China remain largely reactive rather than preventive, with an overreliance on vaccines at the expense of non-pharmaceutical biosecurity measures (Chen et al., 2021). As a result, veterinarians may have a limited understanding and adoption of biosecurity as a structured, preventive approach to disease control.

The current *Brucella* spp. control policies were supported by most participants in Henan Province, but with limited confidence in achieving *Brucella* spp. control and elimination (Figure 4.2). This distinction is complicated by multiple factors such as the perception of disease, budgets, and insufficient government compensation. Denis-Robichaud et al. (2019b) suggested that perceiving the financial benefits and effectiveness of implementing control measures, such as farm biosecurity practices and culling test-positive animals, is difficult for farmers, especially for animals that appear to be ‘apparently healthy’ and ‘productive’ (Kristensen et al., 2011; Zhu et al., 2023a). Our survey results also support the hypothesis that less than half of the respondents believed that *Brucella* spp. control and elimination improve cattle productivity, milk quality, and price. Economic costs remain the most significant challenge to implementing the *Brucella* spp. control and elimination program for dairy farmers (McDermott et al., 2013; Zhang et al., 2018). In China, compensation for the compulsory culling of *Brucella*-positive animals well below market value is the primary reason farmers tend not to comply with current policies. There is a need to increase compensation and incentives and provide scientific and quantitative economic return statistics to encourage dairy farmers to adopt farm biosecurity practices and other control measures.

A structured and closed-questionnaire approach was selected to assess farm biosecurity practices and farmers’ motivations for brucellosis in Henan dairy herds. This approach was chosen over open-ended questionnaires to enhance data consistency, facilitate statistical analysis, and minimize interviewer bias, but also sacrificed the flexibility and depth of the answers provided by farmers (Dohoo et al., 2009). Given the need for statistical insights into biosecurity adoption patterns, structured questions allowed for direct comparisons between farms and the application of advanced statistical techniques, such as multiple correspondence analysis and hierarchical clustering. However, we acknowledge that closed questionnaire design may limit the depth and diversity of response information gathered. Open-ended questions could provide richer insights into farmers' perceptions of biosecurity policies, their real motivations, and the underlying behavioral drivers influencing their decision-making (Musallam et al., 2015b; Zeng et al., 2018).

While this study provides valuable insights into farm biosecurity practices adoption among Henan dairy herds, some potential biases may impact the findings. The sampling population and questionnaire of this study may not fully capture the diverse range of practices across all dairy herds, especially for non-intensive (N < 100 animals) dairy herds that were not included in the Henan DHI list. The findings in the study may not be applied to these non-intensive dairy herds, and future studies should investigate them. The contacts of non-intensive dairy herds were not accessible to researchers and the public probably due to privacy concerns and no contacts, which emphasize the need for a dairy herd recruitment platform to protect farm privacy and disseminate research recruitment information and findings. Small dairy herds had poor husbandry management (Chen et al., 2021) and were positively associated with brucellosis positivity (odds ratio = 4.6) in China (Ma et al., 2014). The lack of this subset of small herds (N < 100) may have led to an overestimation of biosecurity adoption in this study. Secondly, self-reported data from respondents may also be subject to recall bias or social desirability bias, potentially causing an overestimation of biosecurity practice adoption. On-farm visits and interviews in our study could help reduce potential biases, but triangulation should be employed by incorporating direct researcher observations during farm visits in the future. Given that this study was conducted during the COVID-19 pandemic, access to farm production areas was limited. This posed challenges for direct observation and triangulation, affecting the verification of reported biosecurity practices. Longitudinal monitoring of farms to maintain recommended biosecurity practices is worthy of investigation because farm workers may temporarily behave in a good way to cater to social preferences during the survey period.

4.6 Conclusions

In conclusion, this study provides valuable and novel insights into the profiles of farm biosecurity practices in Henan dairy herds. Identifying distinct clusters and inadequate biosecurity practices offers evidence-based guidance for future farm biosecurity training programs. Moreover, the study highlights the need for improved financial compensation and incentives to motivate farmers' adoption of biosecurity measures. Further research is encouraged to assess the effectiveness and economic benefits of implementing farm biosecurity practices and provide a robust foundation for *Brucella* spp. control and elimination programs in Henan Province, China.

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Chapter 5 Effects of *Brucella* spp. on milk yield, somatic cell count and pregnancy in dairy cows in Henan dairy herds, China

Yu Wang,[1] Emilie Vallée,[1] Cord Heuer,[1] Aizhen Guo,[2] Youming Wang,[3] Zhen Zhang,[4] and Chris Compton[1]

[1]EpiCentre, School of Veterinary Science - Tāwharau Ora, Massey University, Private Bag 11-222, Palmerston North, 4410, New Zealand

[2]National Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China

[3]China Animal Health and Epidemiology Center, Qingdao, 266032, China

[4]Henan Dairy Herd Improvement Center, Zhengzhou, Henan, 450045, China

Preface

The primary driver for dairy farm stakeholders to adopt active disease control measures is the perceived impact of disease, particularly its effects on production and zoonotic risk to humans. Brucellosis significantly affects dairy cattle by increasing abortion rates, reducing milk yield, and contributing to mastitis, leading to substantial economic losses. Chapter 2 found the increasing seroprevalence of brucellosis in the Chinese dairy population from retrieved publications, and Chapter 4 highlighted that stakeholders prioritize dairy cow productivity and financial returns when assessing the required efforts and budget for a disease control program. The magnitude of brucellosis consequences is influenced by a range of factors, including prevailing *Brucella* spp., and population-specific characteristics such as breed and age. However, quantitative estimates of brucellosis-related consequences in dairy cattle remain unknown, both in China and globally. This knowledge gap is likely due to constraints in funding, longitudinal data collection, and data quality. Chapter 5 will figure out these knowledge gaps around disease impacts on production in dairy cattle. Addressing these knowledge gaps can not only directly enhance stakeholders' understanding of disease consequences and the adoption of active measures but also offer robust population-specific parameters for subsequent financial analysis (Chapter 6).

5.1 Abstract

Bovine brucellosis, primarily caused by *Brucella abortus* (*B. abortus*), significantly affects dairy cattle health and productivity worldwide, yet quantitative assessments of disease impacts remain scarce in China and globally. This longitudinal study aimed to investigate the incidence of seroconversion of *Brucella* spp. and its association with daily milk yield (DMY), somatic cell count (SCC), and pregnancy outcomes in dairy cattle. We measured antibodies against *Brucella* spp. in around 100 pregnant cows or heifers in each of twelve Henan dairy herds at their pregnancy testing and again at subsequent pregnancy testing between July 2021 and October 2022. Serum samples were tested in series using the Rose Bengal Test and Competitive ELISA to determine *Brucella* spp. status at the animal level. Productivity data were collected from the Henan Dairy Herd Improvement Center database and farmers. Generalized Additive Mixed Models and Generalized Estimating Equations were employed to estimate associations between disease status and productivity outcomes. The study found an overall seroprevalence of 13.6% (95% CI: 11.7, 15.7) and 23.1 (95% CI: 20.6, 25.7) at two testing points, respectively, resulting in a seroconversion of incidence at 13.1% (95% CI: 10.9, 15.6) over one year with a range between 0.0% and 24.3%. Seroconversion negatively impacted DMY, with consistently negative cows producing an average of 3.2 kg (95% CI: 2.4, 4.0) and 2.8 kg (95% CI: 2.0, 3.6) more milk per day than seroconverted cows and consistently positive cows, respectively. Elevated SCC was significantly associated with seroconversion, indicating potential impacts on milk quality. A positive association between seroconversion to *Brucella* spp. and pregnancy loss was identified, with relative risk at 4.26 (95% CI: 3.17, 5.73). This study provided essential epidemiological insights into the seroconversion of *Brucella* spp. and its implications on three essential dairy productivity outcomes. The impacts of disease on production highlight the need for strategic interventions to improve the health, productivity, and economic sustainability of dairy farming in China and brucellosis-endemic regions.

Keywords: *Brucella* spp., seroconversion, milk yield, somatic cell count (SCC), abortion, multilevel modeling

5.2 Introduction

Bovine brucellosis, primarily caused by *Brucella abortus* (*B. abortus*), is a significant zoonotic disease affecting the productivity and welfare of dairy cattle and human health (Moriyón et al., 2023). While some developed nations, such as Australia and New Zealand, have successfully eliminated the disease, it remains prevalent in most developing countries, resulting in substantial economic burdens and health challenges (Moriyón et al., 2023; Zhang et al., 2018). Given the notable elevation in the prevalence of brucellosis in dairy cattle and incidence in humans in China (Lai et al., 2017; Ran et al., 2019; Wang et al., 2021b), government and farm stakeholders endeavor to adopt more stringent control programs to contain this disease. *B. melitensis* has been identified as the primary causative agent of human brucellosis, and *B. abortus* accounted for most cases in dairy cows in China (Wang et al., 2024b). Cattle infected with *Brucella* spp. typically remain asymptomatic until the first gestation, displaying clinical signs such as abortion, reduced milk yield, temporary or permanent infertility, and arthritis (Bercovich, 1998; Neta et al., 2010). Beyond these tangible manifestations, the invisible impacts extend to mastitis, premature culling, and costs associated with prevention and control measures, collectively resulting in significant economic losses for farmers (Bernués et al., 1997; Kiiza et al., 2023; Singh et al., 2018). Transmission within herds primarily occurs through direct contact with infected cattle and contaminated products (e.g., aborted fetuses, placenta), and the consumption of raw milk (Moriyón et al., 2023; Zhang et al., 2018).

Milk production and pregnancy success are essential for sustainable development and economic viability for dairy farms, but they are closely linked to the incidence of infectious diseases such as brucellosis (Kiiza et al., 2023). Understanding the impact of brucellosis on milk production and pregnancy success is crucial for motivating the adoption of control measures and resource allocation (Chen et al., 2021). Despite many studies identifying the association of seropositivity to *Brucella* spp. with abortion in various livestock species, including dairy cattle, these have predominantly employed cross-sectional designs (Holt et al., 2021; Zeng et al., 2017; Zhang et al., 2020). Such designs can introduce potential biases by failing to establish the temporal relationship between seropositivity and productivity loss (Wang et al., 2022). Interestingly, two studies have reported no decrease or even an increase in milk production for seropositive cows (Mellado et al., 2014; Mellado et al., 2021), while brucellosis is traditionally regarded as reducing milk yield (Bernués et al., 1997). Evaluating the effects of *Brucella* spp. on productivity in dairy cows requires long-term observation and consideration of context-specific management and environment, resulting in few studies reporting it in China and worldwide.

Economic studies have highlighted milk yield losses and increased incidence of abortion as substantial contributors to overall estimations of the financial impact of brucellosis (Kiiza et al., 2023; Singh et al., 2015; Singh et al., 2018). However, these estimations require population-specific empirical data to derive accurate milk and pregnancy loss estimates. Additionally, *Brucella*-related mastitis, characterized by multifocal interstitial inflammation, may contribute to increased somatic cell count (SCC) in milk (Capparelli et al., 2009; Neta et al., 2010). Despite the crucial role of SCC as an indicator of mastitis in dairy cows and its significant influence on milk quality and monetary value (Hadrich et al., 2018), no studies have explored the association between *Brucella* exposure status and SCC at the animal level. Factors such as parity (lactation), days in milk, herd size, and sampling season may be potential confounders for exploring the effects of *Brucella* exposure status on milk production, SCC, and pregnancy loss (Hagnestam-Nielsen et al., 2009; Mellado et al., 2014; Mellado et al., 2021).

Given the identified research questions, this prospective longitudinal study aimed to estimate

(1) the seroprevalence of and incidence of seroconversion to *Brucella* spp. and (2) the association between seroconversion to *Brucella* spp. and three productive outcomes – DMY, SCC, and pregnancy loss in dairy cattle in Henan Province, China. Insights gained from this study could enhance the understanding of disease impact on productivity and inform prioritized disease control strategies for policymakers and farms in China and other affected countries.

5.3 Materials and Methods

5.3.1 Study design and sample collection

The study adhered to the STROBE-Vet reporting guidelines (Sargeant et al., 2016) for data analysis and reporting. The study was conducted in the Henan Province of China, which hosted approximately 385,000 dairy cattle and produced 2.17 million tons of dairy products in 2021 (National Bureau of Statistics of China, 2023). The dairy farming landscape in Henan is characterized by over 90% of the dairy population residing in approximately 150 dairy herds under intensive management within free stall housing pens. The Holstein-Friesian is the predominant breed, with semen primarily sourced from provincial breeding bull stations and commercial breeding enterprises. Nearly all intensive dairy herds (N >100) participated in the Henan Dairy Herd Improvement (DHI) program, which provides services, including monthly milk composition monitoring, early pregnancy diagnosis, disease diagnosis, and feed component testing.

A longitudinal study was conducted between July 2021 and October 2022 to collect samples and epidemiological data from local dairy herds and the Henan DHI Centre. A list of dairy herds and contacts was supplied by the Henan DHI Centre, combined with the brucellosis vaccination history of individual dairy herds obtained from the Henan Province Center for Animal Disease Control and Prevention. Twelve dairy herds were selected based on the following criteria: (1) willingness to provide serum samples from early pregnancy diagnosis for this study; (2) maintenance of good production and DHI records; (3) no vaccination against *Brucella* within the last three years. Specific sampling details and essential herd characteristics are shown in Table S 4.1 of Appendix 4. Blood samples collected during routine early pregnancy testing 28 days after artificial insemination were obtained from approximately 100 randomly selected cows in each herd between July 2021 and November 2021. These samples were initially intended for pregnancy testing and were re-purposed in this study. Follow-up samples were collected from the same population by unique identification numbers between July 2022 and October 2022. Reasons for follow-up losses were ascertained through subsequent phone contacts with farmers.

The sample size for this study was calculated based on an assumed power of 0.8, a 95% confidence level, an 8% incidence of abortion in unexposed cows, and a relative risk of 3.8 for abortion in seroconverted or consistently positive cows compared to consistently negative cows. This generated a minimum sample size of 90 for each herd. After adjusting for a 10% follow-up loss, the final sample size was 99 animals per herd. The calculation was implemented through the epiR package version 2.0.65 (Stevenson et al., 2022). The 8% incidence of abortion was estimated based on consultation with the staff of the Henan DHI Centre, while the relative risk of 3.8 was derived from published literature (Etefa et al., 2022).

5.3.2 Diagnostic tests

Approximately 5 ml of blood samples were collected from the caudal veins of dairy cows, labeled, and transported to the Henan DHI Center within 24 hours in an ice box. Serum samples were separated by centrifugation at 3,000 rpm for 10 minutes and stored at -20°C until testing, with a maximum storage period of 12 months. Each serum sample was initially tested with a commercial Rose Bengal Test (RBT) (Harbin Pharmaceutical Group Bio-vaccine Co., Ltd, China), with positive samples followed by a commercial C-ELISA (Ingezim *Brucella* Compac 2.0, Spain) kit. The sensitivity and specificity of C-ELISA in dairy cows have been previously evaluated as 81.6% and 97.1%, respectively, at the optimal cutoff value of 0.403, and for the RBT, 79.9% and 99.6%, respectively (Wang et al., 2024a), generating a combined sensitivity (65.2%) and specificity (99.9%). Samples were classified as positive if both tests were positive and negative otherwise.

5.3.3 Data collection and definitions

Data including daily milk yield (DMY, kg/day), somatic cell count (SCC, 1,000 cells/mL), lactation (parity), days in milking (DIM, day), dates of important activities (calving, artificial insemination, and sampling), pregnancy outcomes (abortion or calving) and herd size were sourced from the Henan DHI Centre database and direct communication with farmers. DMY and SCC were recorded once a month. Herd size was categorized as large (>2000), medium (900-2000), and small (<900). Lactation was classified into four categories (1^{st}, 2^{nd}, 3^{rd}, and $\geq 4^{th}$), and seasons were defined as spring (March-May), summer (June-August), autumn (September-November), and winter (December-February). Observations with DIM ≥ 400 days

(7.8%) or SCC ≥ two million cells/mL (2.0%) were excluded from further data analysis due to their deviations from the normal lactation range (personal communication with Henan DHI Centre) and potential inaccuracies for these extreme values (Hand et al., 2012). *Brucella* spp. exposure status was categorized into three groups: (1) consistently negative, (2) consistently positive, (3) seroconverted from negative to positive. Few animals (0.8%) that seroconverted from positive to negative to *Brucella* spp. were discarded from further analysis.

5.3.4 Statistical analysis

Data were recorded in Excel spreadsheets and analyzed using R programming software version 4.1.2 (R Core Development Team, 2021). Descriptive statistics were presented for categorical variables as counts and percentages (N and %) and continuous variables as median and interquartile range (IQR) or mean and standard deviation (SD). Continuous variables (DMY and SCC) were checked for normality through plotting histograms, and SCC was log-transformed to achieve approximate normality.

Seroprevalence and 95% Wilson confidence intervals (95% CI) were calculated at each sampling point. The incidence of seroconversion to *Brucella* spp. was defined as the proportion of animals transitioning from seronegative to seropositive against *Brucella* spp. between two sampling points among those seronegative animals at initial sampling. The interval between two sampling points was incorporated into the following analysis as a potential confounder. DIM was an essential factor influencing outcome variables, and the relationship between DIM and outcome variables did not comply with the linearity assumption based on visualization examination.

Generalized Additive Mixed Models (GAMMs) were used to estimate associations between continuous outcome variables (DMY and log-transformed SCC) and explanatory variables. This statistical approach allowed the exploration of flexible and nonlinear relationships between outcome and explanatory variables and has been widely applied in dairy science (Bonestroo et al., 2022; Huang et al., 2023). Cubic regression splines with shrinkage were applied using restricted maximum likelihood (REML) to model the smoothing effect of days in milking, stratified by the variable of *Brucella* spp. exposure status. This approach allows for *Brucella* spp. status-specific smoothing. Random effects at individual and herd levels were introduced to account for potential clustering within milking cows and herds. Explanatory variables included exposure status, the interval between testing points, lactation, DIM, season, and herd size. Univariable models were initially employed, with variables demonstrating a p-value < 0.20 progressing to multivariable models. The final model underwent examinations for multicollinearity using variance inflation factors (VIF), with variables exceeding VIF > 5 being removed. When more than two variables had VIF > 5, the variable with the highest VIF was removed first unless it was epidemiologically crucial, followed by checking pairwise correlations, removing the least informative variable, and iteratively recalculating VIF. Interactions between predictor variables were examined for statistical significance, with only those having a p-value < 0.05 retained. The intraclass correlation coefficient (ICC) was calculated to explore the proportion of variance explained by the animal-level and herd-level random effects in the data (Dohoo et al., 2009). Residuals were assessed for normality, homoscedasticity, and autocorrelation by plotting. Variables with a p-value < 0.05 were retained in the final model. Two continuous outcome variables (DMY and SCC) were predicted based on developed GAMMs stratified by *Brucella* spp. exposure status and lactation. The "mgcv" package version 1.9-0, "gamm4" package version 0.2-6, and "ggeffects" package version 1.3.2 (Lüdecke, 2018) were used in model development and predictions (Wood, 2017).

A log-binomial generalized estimating equation (GEE) with an exchangeable correlation structure was used to estimate the association between the occurrence of abortion and *Brucella* spp. exposure status. The GEE modeling approach considered the potential clustering effect within herds by introducing a herd-level grouping term in the model. Explanatory variables included parity, *Brucella* spp. exposure status, herd size, and the interval between two testing points. Variables with a p-value < 0.20 in the univariable GEE models were subjected to multivariable GEE models. A confounder, defined as altering other coefficients by more than 15%, was forcibly retained in the final model irrespective of its significance (Dohoo et al., 2009). Variables with a p-value < 0.05 were retained in the final model, and interaction terms between variables were tested and included if the p-value was < 0.05. The quasi-likelihood information criterion (QIC) was calculated to compare models, with lower QIC values indicating a better fit. The risk of abortion and its 95% CI were calculated by *Brucella* spp. exposure status and lactation. The "geeglm" package version 1.3.9 (Højsgaard et al., 2006) and "ggeffects" package version 1.3.2 in R were used for GEE model development and predictions. The package "tidyverse" version 2.0.0 was used for data cleaning and visualization (Wickham et al., 2019).

5.3.5 Ethics approval

The study protocol was approved by a local ethics committee – Animal Experimental Ethical Inspection of Laboratory Animal Centre, Huazhong Agricultural University (Protocol Number: HZAUBU-2021-0003). The study involving human participants was reviewed and approved by peer review at Massey University Human Ethics Committee (Protocol Number: SOA 21/60). The written consent of farm owners was secured before blood samples were collected from dairy farms.

5.4 Results

5.4.1 Prevalence and incidence of seroconversion to *Brucella* spp.

A total of 1154 cattle from twelve Henan dairy herds were initially included in the study, with 1080 animals participating in the follow-up test, resulting in a 6.4% follow-up loss. Most farms (11/12) met the minimum sample size criteria, except for one farm, where only 25 samples were collected due to limited animals for artificial insemination and early pregnancy testing in this farm. The average interval between the two testing points was 368.0 days (IQR: 356.0, 379.0). Test prevalence of *Brucella* spp. among twelve selected dairy herds ranged from 0% (95% CI: 0.0, 3.6) to 33.6% (95% CI: 25.4, 43.0) at the first test, with an overall prevalence of 13.6 (95% CI: 11.7, 15.7) (Table 5.1). During the follow-up testing, visible increases in test prevalence were observed in 9/12 herds (Range: 19.6%-43.5%, Table 5.1). Of the 946 animals that initially tested negative, 124 seroconverted from negative to positive against *Brucella* spp., generating a test incidence of 13.1% (95% CI: 10.9, 15.6) over one year. Specific incidence of seroconversion to *Brucella* spp. varied between 0.0% and 24.3% across herds (Table 5.1). After excluding three herds where no positive animals were identified in both tests, the incidence of seroconversion increased to 18.9% (95% CI: 15.7, 22.5), significantly higher than the overall estimate (Pearson $\chi^2 = 9.86$, p-value < 0.01). 11.6% of animals remained positive in both tests, while 0.8% transitioned from seropositive to seronegative, which were discarded from subsequent analyses.

Among the 6.4% follow-up losses population, the animal-level seroprevalence was 31.1% (95% CI: 21.7, 42.3) at the initial test, significantly higher than that of the successfully traced population (13.6%, 95% CI: 11.7, 15.7, Pearson $\chi^2 = 17.0$, p-value < 0.001) with a prevalence ratio of 2.3 (95% CI: 1.6, 3.3). Causes for follow-up loss are detailed in Table S 4.2 of Appendix 4, and the longitudinal serological test results are summarized in Table S 4.3 of Appendix 4. Animals present on the farm but not captured in the second round of testing were the top reason (18/74), followed by mastitis (16/74) and low milk production (15/74).

Table 5. 1 Overview of test results of prevalence and incidence of seroconversion to *Brucella* spp. and their 95% confidence intervals (CI) in twelve dairy farms in Henan Province between July 2021 and October 2022

Farm	1st test (P/N[a])	Prevalence (95% CI), %	2nd test (P/N[a])	Prevalence (95% CI), %	Seroconversion (S/N[b])	Incidence (95% CI)
1	36 / 107	33.6 (25.4 – 43.0)	36 / 97	37.1 (28.2 – 47.0)	7 / 66	10.6 (4.3 – 21.9)
2	9 / 100	9.0 (4.8 – 16.2)	18 / 92	19.6 (12.7 – 28.8)	11 / 85	12.9 (6.5 – 23.2)
3	2 / 25	8.0 (2.2 – 25.0)	7 / 25	28.0 (14.3 – 47.6)	5 / 23	21.7 (7.1 – 50.7)
4	29 / 113	25.7 (18.5 – 34.4)	37 / 104	35.6 (27.0 – 45.1)	17 / 82	20.7 (12.1 – 33.2)
5	9 / 99	9.1 (4.9 – 16.4)	29 / 97	29.9 (21.7 – 39.6)	21 / 88	23.9 (14.8 – 36.5)
6	10 / 102	9.8 (5.4 – 17.1)	27 / 98	27.6 (19.7 – 37.1)	19 / 90	21.1 (12.7 – 33.0)
7	22 / 100	22.0 (15.0 – 31.1)	35 / 93	37.6 (28.5 – 47.8)	18 / 74	24.3 (14.4 – 38.4)
8	0 / 103	0.0 (0.0 – 3.6)	0 / 97	0.0 (0.0 – 3.8)	0 / 97	0.0 (0.0 – 3.8)
9	33 / 99	33.3 (24.8 – 43.1)	40 / 92	43.5 (33.8 – 53.7)	12 / 62	19.4 (10.0 – 33.8)
10	0 / 101	0.0 (0.0 – 3.7)	0 / 96	0.0 (0.0 – 3.8)	0 / 96	0.0 (0.0 – 3.8)
11	0 / 102	0.0 (0.0 – 3.6)	0 / 96	0.0 (0.0 – 3.8)	0 / 96	0.0 (0.0 – 3.8)
12	7 / 103	6.8 (3.3 – 13.4)	20 / 93	21.5 (14.4 – 30.9)	14 / 87	16.1 (8.8 – 27.0)
Overall	157 / 1154	13.6 (11.7 – 15.7)	249 / 1080	23.1 (20.6 – 25.7)	124 / 946	13.1 (10.9 – 15.6)

Note: [a]: number of positive/number tested; [b]: number seroconverted/number of test-negative animals at the initial test

5.4.2 Effects of *Brucella* spp. exposure status on daily milk yield (DMY)

Observational data suggested that the consistently negative group exhibited a higher average unadjusted DMY (29.6 kg/day, SD = 9.6) compared to the seroconverted group (26.6 kg/day, SD = 6.9) and consistently positive group (26.1 kg/day, SD = 7.7) (Table 5.2). A scatter plot for DMY was created to show its trend along with DIM, stratified by *Brucella* spp. exposure status (Figure S 4.1). Four predictor variables (*Brucella* spp. exposure status, lactation, season, and herd size) and one smoothing term (DIM) were associated with DMY in the univariable analysis and were subsequently offered to multivariable analysis (Table 5.3). Cows in medium and large-sized herds produced more milk than those in small-sized herds (coefficients = 3.06 and 1.12, respectively, both *p*-values < 0.05), and milk yield was higher in summer and autumn than in spring (coefficients = 0.77 and 0.45, *p*-values < 0.05). Seroconverted cows produced the least DMY (coefficient = -3.20, 95% CI: 2.4, 4.0, *p*-value < 0.001) compared to consistently negative cows, followed by consistently positive cows (coefficient = -2.80, 95% CI: 2.0, 3.6, *p*-value < 0.001, Table 5.3 and Figure 5.1). The DMY of third-lactation cows was 4.14 kg per day significantly higher than that of first-lactation cows on average, followed by fourth or more lactation cows (coefficient = 3.20, *p*-value < 0.001) and second-lactation cows (coefficient = -2.00, *p*-value < 0.001). Predicted lactation curves, stratified by exposure status and lactation and adjusted for spring and medium herd size, revealed that DMY were significantly higher in consistently negative cows than in seroconverted and consistently positive cows regardless of lactations (Figure 5.1), and the gap between DMY in the peak lactation period is greater than that in the early and late lactations (Figure 5.1). There was no apparent difference in predicted average DMY between the seroconverted group and the consistently positive. DMY was mainly clustered at the cow level (ICC at cow level = 0.243) and less at the herd level (ICC at herd level = 0.015).

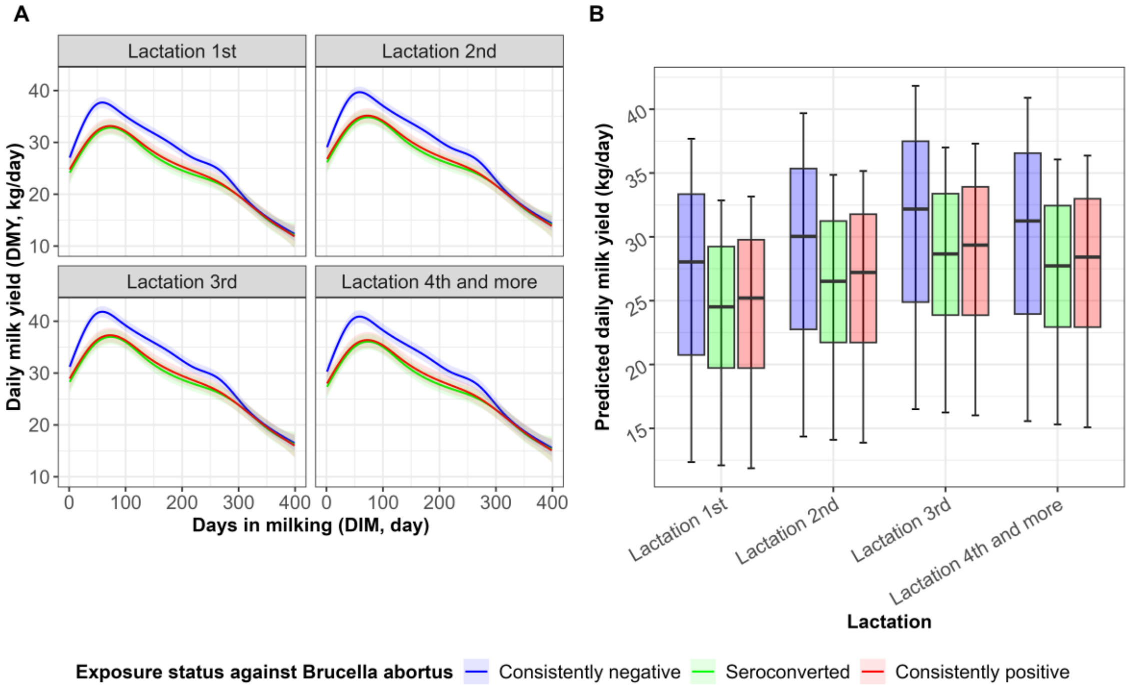

Figure 5. 1 Predicted daily milk yield (DMY, kg/day) from multivariable GAMM, stratified by lactation and exposure status against *Brucella* spp. (A) Predicted lactation curve; (B) boxplot of the distribution of DMY predictions over a lactation

Table 5. 2 Descriptive statistics of daily milk yield (kg/day) after excluding extreme values (days in milking more than 400 days, somatic cell count more than 2000 thousand cells/mL), stratified by exposure status against *Brucella* spp.

Category of exposure status	Number of observations	The mean number of observations per cow	Average daily milk yield (kg/day)	Standard deviation	Median	Range (Min, Max)
Consistently negative group	6414	7.9	29.6	9.6	29.1	1.9, 78.2
Seroconverted group	1024	8.3	26.6	6.9	26.5	0.3, 53.9
Consistently seropositive	1036	8.4	26.1	7.7	25.8	7.5, 58.9
Overall	8474	8.1	28.8	9.2	28.5	0.3, 78.2

Table 5. 3 A generalized additive mixed model (GAMM) to estimate the effect of exposure status to *Brucella* spp. on daily milk yield (DMY).

Variables	Category	Coefficient	Standard error	T value	*p*-value
Intercept		25.5	0.52	49.10	< 0.001
Exposure status	Consistently negative	Reference			
	Seroconverted	-3.20	0.42	-7.64	< 0.001
	Consistently positive	-2.80	0.42	-6.61	< 0.001
Lactation	1st	Reference			
	2nd	2.00	0.25	8.15	< 0.001
	3rd	4.14	0.31	13.30	< 0.001
	4th and more	3.21	0.43	7.43	< 0.001
Herd size	Small	Reference			
	Medium	3.06	0.64	4.75	< 0.001
	Large	1.12	0.64	1.75	0.081
Season	Spring	Reference			
	Summer	0.77	0.20	3.82	< 0.001
	Autumn	0.45	0.21	2.134	0.033
	Winter	0.003	0.20	0.016	0.988
S(Days in milking) : consistently negative		Edf = 8.803, ref.df = 9, F = 575.31, *p*-value < 0.001			
S(Days in milking) : seroconverted		Edf = 7.393, ref.df = 9, F = 73.63, *p*-value < 0.001			
S(Days in milking) : consistently positive		Edf = 7.273, ref.df = 9, F = 91.04, *p*-value < 0.001			
		Adjusted R-squared = 0.559			

ICC at cattle level = 0.243, ICC at herd level = 0.015, the proportion of variance at cattle level = 0.228, the proportion of variance at lactation level = 0.757. S: Smoothing method, cubic regression spline with shrinkage.

5.4.3 Effects of *Brucella* spp. exposure status on somatic cell count (SCC)

Observational data indicated that the consistently negative group (median = 109, 1000 cells/mL) displayed a significantly (Wilcoxon test p-values < 0.001) lower median unadjusted SCC compared to the seroconverted (median = 244, 1000 cells/mL) and consistently positive (median = 234, 1000 cells/mL) groups (Table 5.4). A scatter plot for log-transformed SCC was created to show its trend along with DIM, stratified by *Brucella* spp. exposure status (Figure S 4.2). Four fixed effect variables (exposure status, lactation, season, and herd size) and one smoothing term (DIM) were significantly associated with log-transformed SCC in the univariable analysis. Subsequent analysis excluded herd size and season, as they were either statistically insignificant or failed to improve model fitness. Interaction terms were examined and discarded due to statistical non-significance. Log-transformed SCC was significantly elevated in seroconverted and consistently positive cows (coefficients = 0.56 and 0.60, p-values < 0.001) compared with consistently negative cows and increased substantially with higher lactation (Table 5.5). The average log-transformed SCC in cows with third lactation and fourth or above lactation was significantly higher than that in cows with 1st lactation (coefficients = 0.26 and 0.52, p-values < 0.001). The non-parametric smoothing term (DIM) was interpreted by plotting them stratified by exposure status and lactation, revealing a nonlinear curve in predicted SCC with DIM for consistently negative cows and a nearly linear increase for seroconverted and consistently positive cows (Figure 5.2). Log-transformed SCC exhibited greater clustering at the cow level (ICC at the cow level = 0.209) than at the herd level (ICC at the herd level = 0.023) (Table 5.5).

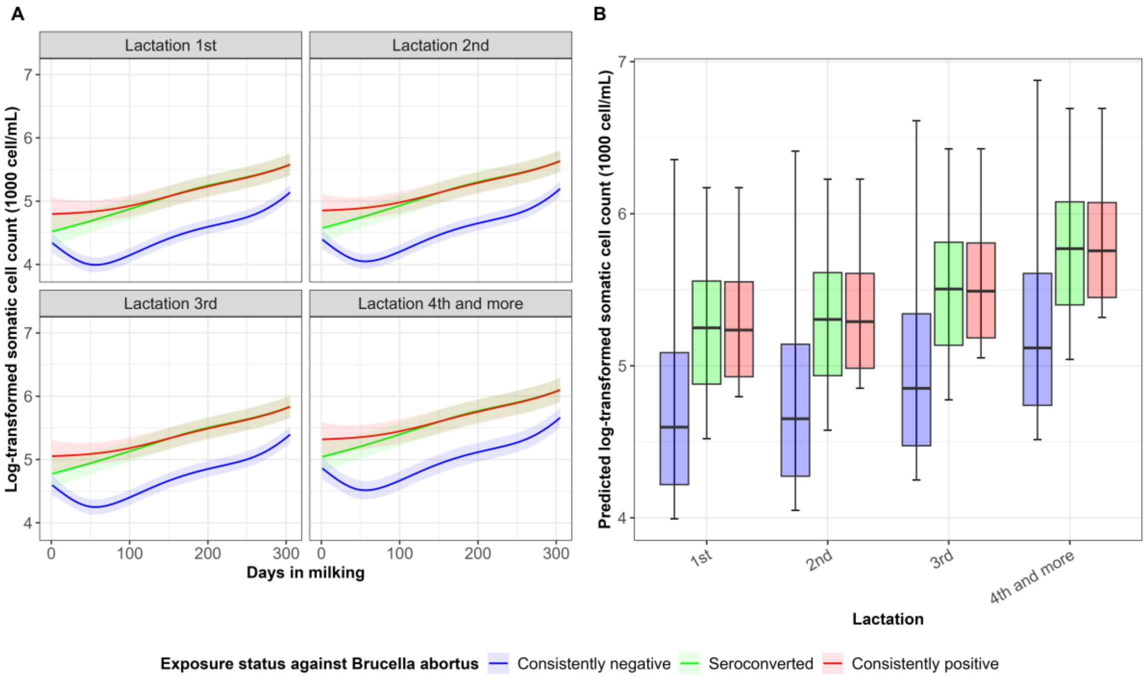

Figure 5. 2 Predicted somatic cell count (SCC, 1,000 cells/mL) from multivariable GAMM, stratified by lactation and exposure status against *Brucella* spp. (A) relationship between days in milking and predicted SCC; (B) boxplot of the distribution of predicted SCC over a lactation.

5.4.4 Effects of *Brucella* spp. exposure status on pregnancy loss

The overall incidence of pregnancy loss throughout the observation period was 10.6% (95% CI: 8.7, 12.8). Seroconverted cows displayed a higher incidence of abortion (29.8%, 95% CI: 21.0, 41.1) compared with consistently negative group (6.3%, 95% CI: 4.7, 8.3) and consistently positive group (19.7%, 95% CI: 12.6, 29.3) (Table 5.6). The time interval between the two testing points was excluded from further multivariable analysis due to its lack of association with pregnancy loss (*p*-value = 0.77). Herd size was partially associated with abortion occurrence in the multivariable analysis (*p*-value = 0.085), then it was removed to simplify the model with limited impacts on coefficients of other variables (< 6.4%). The interaction term between *Brucella* spp. exposure status and lactation was dropped because incorporating it increased the model complexity but presented a poorer fitting with a larger QIC (QIC with interaction term = 655.5 venus QIC without interaction term = 653.8). The final multivariable model was built with *Brucella* spp. exposure status and lactation (Table 5.7). Seroconverted (RR = 4.26, 95% CI: 3.17, 5.73) and consistently positive cows (RR = 3.25, 95% CI: 2.39, 4.42) exhibited a higher risk of abortion over one year compared to consistently negative cows (Figure 5.3 Panel A). The risk of abortion generally increased with the lactation of cows and was significantly higher in cows with lactation 3rd (RR = 1.51, 95% CI: 1.07, 2.12) and lactation 4th and above (RR = 2.08, 95% CI: 1.33, 3.27) relative to primiparous heifers. A dot plot (Figure 5.3 Panel B) was drawn to visualize the predicted probability of abortion over one year at each parity and *Brucella* spp. exposure status based on the final model (Table 5.7). The ICC at the herd level was calculated at -0.0029, indicating no significant clustering at the herd level.

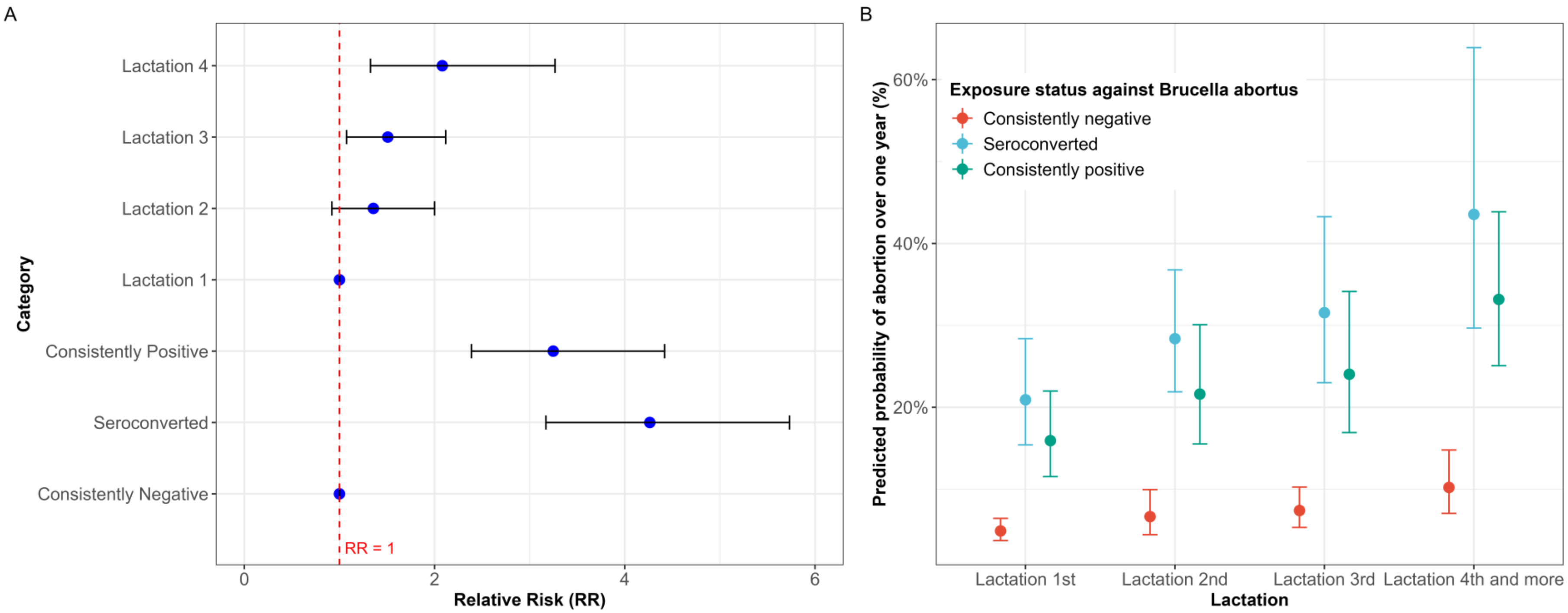

Figure 5. 3 Barplots shows the relative risk of each category and 95% confidence interval (Panel A) and predicted probability of abortion over one year (Panel B), stratified by lactation and exposure status against *Brucella* spp.

Table 5. 4 Descriptive statistics of somatic cell count (SCC, 1000 cells/mL) after excluding extreme values (days in milking more than 400 days, somatic cell counts more than 2000 thousand cells/mL), stratified by exposure status against *Brucella* spp.

Category of exposure status	Number of observations	Average number of observations per cow	Average SCC	Standard deviation	Median SCC	IQR1, IQR3
Consistently negative group	6414	7.94	197	259	109	52, 228
Seroconversion group	1024	8.33	356	350	244	130, 449
Consistently seropositive	1036	8.35	340	334	234	121, 435
Overall	8474	8.12	134	289	233.3	61, 279

IQR: Interquartile range

Table 5. 5 A generalized additive mixed model (GAMM) to estimate the effect of seroconversion to *Brucella* spp. on log-transformed somatic cell count (SCC)

Variables	Category	Estimate	SE	T value	*p*-value
Intercept		4.60	0.05	88.85	< 0.001
Exposure status	Consistently negative	Reference			
	Seroconverted	0.56	0.06	9.64	< 0.001
	Consistently positive	0.60	0.06	10.19	< 0.001
Lactation	1st	Reference			
	2nd	0.06	0.04	1.53	0.126
	3rd	0.26	0.05	5.66	< 0.001
	4th	0.52	0.06	8.42	< 0.001
S(Days in milking) : consistently negative			Edf = 7.24, ref.df = 9, F = 187.7, $p < 0.001$		
S(Days in milking) : seroconverted			Edf = 3.13, ref.df = 9, F = 17.4, $p < 0.001$		
S(Days in milking) : consistently positive			Edf = 3.15, ref.df = 9, F = 15.1, $p < 0.001$		
			Adjusted R-squared = 0.263		

ICC at cattle level = 0.209, ICC at herd level = 0.023, the proportion of variance at cattle level = 0.186, and the proportion of variance at lactation level = 0.791. S: Smoothing method, cubic regression spline with shrinkage.

Table 5. 6 Incidence of pregnancy loss and their 95% confidence intervals (CI), stratified by exposure status against *Brucella* spp.

Category of exposure status	Number of pregnancy loss	Number of cattle at pregnancy	Incidence of pregnancy loss (%)	Confidence interval (%)
Consistently negative group	51	808	6.3	4.7, 8.3
Seroconversion group	37	124	29.8	21.0, 41.1
Consistently seropositive	24	122	19.7	12.6, 29.3
Overall	112	1054	10.6	8.7, 12.8

Table 5. 7 Multivariable generalized estimating equation (GEE) to estimate the effect of exposure status against *Brucella* spp. on the occurrence of abortion

Variables	Category	Estimate	Standard error	Wald value	Relative risk (95% CI)	*p*-value
Intercept		-3.02	0.14	470.41		< 0.001
Seroconversion status	Consistently negative	Reference				
	Seroconverted	1.45	0.15	92.59	4.26 (3.17, 5.73)	< 0.001
	Consistently positive	1.18	0.16	56.22	3.25 (2.39, 4.42)	< 0.001
Lactation	1st	Reference				
	2nd	0.31	0.20	2.38	1.36 (0.92, 2.00)	0.123
	3rd	0.41	0.17	5.64	1.51 (1.07, 2.12)	0.018
	4th	0.73	0.23	10.20	2.08 (1.33, 3.27)	0.001

5.5 Discussion

This study investigated the seroconversion of *Brucella* spp. and its effects on DMY, SCC, and abortion over one year in dairy cattle in Henan Province, China. Our findings provided empirical evidence that *Brucella* spp. exposure negatively affects milk production, mammary gland health, and reproductive outcomes, emphasizing its significance as a production-limiting disease. Such evidence provides valuable epidemiological insights into the disease impacts on productivity, guiding evidence-based benchmarks for financial analysis and disease control priorities of *Brucella* spp. in dairy cattle in China and other endemic areas.

The estimated incidence of seroconversion of *Brucella* spp. was 13.6% (95% CI: 11.7, 15.7) over one year in this study, lower than estimates reported in other studies (Lambert et al., 2022; Njeru et al., 2021; Wang et al., 2020c), ranging between 11.5% and 33.6% per year. This variation might reflect differences in husbandry production systems, population density, biosecurity, replacement rate, host species, population susceptibility, herd immunity, and prevalent *Brucella* spp. The distinction between intensive and pastoral farming systems has been well-documented in affecting brucellosis transmission (Njeru et al., 2021), with intensive farms often having stronger farm biosecurity but higher animal contact rates (Shortall et al., 2017), whereas pastoral settings have reduced farm biosecurity yet lower contact frequencies (Zeng et al., 2018). High replacement rates may increase the likelihood of animals being prematurely eliminated before testing, resulting in an underestimated incidence, while low replacement rates have less impact if testing frequency is sufficiently short (Dieste-Pérez et al., 2016). The follow-up loss of 6.4% (74/1154) may underestimate the incidence of seroconversion as the follow-up loss group already showed a higher prevalence (31.1% - 23/74) than the remaining population. In addition, the small number of cows (0.8%) that changed from positive to negative at consecutive tests may have been due to poor test sensitivity (65.2% for RBT and C-ELISA in series) used in this study and the natural decay of antibodies. In the Chinese dairy farming system, only one study investigated the incidence risk of brucellosis at 33.2% per year in intensively managed dairy farms in Hubei Province (Wang et al., 2020c), and one abortion outbreak occurred during this study period and likely contributed to a higher incidence (Wang et al., 2022). However, these are different populations located in different provinces of China (Wang et al., 2020c; Wang et al., 2022), which cannot be directly compared given there are many uncertain factors that could lead to variations in disease incidence.

Brucellosis has detrimental consequences on the productivity of dairy cows (Herrera et al., 2008), which are significant concerns motivating farmers to adopt countermeasures in China and other countries (Zeng et al., 2019a; Zhang et al., 2018). Seroconverted and consistently positive cows produced 3.2 and 2.8 kg less milk per day, respectively than consistently negative cows. This aligns with previous findings indicating 10%–25% reductions in milk yield due to brucellosis (Bernués et al., 1997). Notably, milk yield reductions were more pronounced during peak lactation (Figure 5.1), elaborating the lactation kinetics and the variable effect of *Brucella* spp. on milk production across an entire lactation. The effect is likely to be amplified during peak lactation and attenuated during off-peak lactation (Smith et al., 2009). In addition, the lack of difference in DMY between seroconverted and consistently positive cows may be attributed to the chronic nature of *Brucella* spp. (Bercovich, 1998; Neta et al., 2010), and chronic carriers may continue shedding bacteria intermittently, maintaining within-herd transmission risks (Capparelli et al., 2009). *Brucella* spp. can induce long-term or even lifelong consequences on productivity in affected cows, aligning with existing literature that highlighted the complex progression of brucellosis and its potential to impair lifelong productivity in dairy cows (Kiiza et al., 2023; Neta et al., 2010; Singh et al., 2015). Given that brucellosis could induce subclinical infections with delayed or prolonged impacts on milk production, these findings underscore the need for adopting active interventions to mitigate disease buden on dairy herds.

Mellado et al. (2014) estimated the effect of brucellosis on milk yield among vaccinated dairy cows, which introduced a systematic bias regarding disease impacts in vaccinated and non-vaccinated animals. Another study, although indicating parity and calving season as significant confounders, did not report them in detail (Mellado et al., 2021), compromising the robustness of the results for further review. A six-year cohort study in a Mexican dairy farm found significant increases in mean daily milk yield and 305-day total milk yield, but limited details hindered thorough understanding without considering essential confounding factors (e.g., parity, DIM) at the animal level and enhanced productivity related to genetic improvement and farming management over a six-year observation period (Herrera et al., 2008). Our study provides additional information by incorporating seroconversion status, lactation, and milking dynamics over whole lactation at the individual animal level, allowing for a more robust assessment of causality. Parental genetic potential for milk production and their *Brucella* spp. status were not available in this study but were likely essential confounders (Szyda et al., 2007). Given just one year observation period, the bias caused by this genetic potential is considered minor. The

Brucella infection status of the parents is worth investigating since positive dam are more likely to produce positive or latent infected offspring. Incorporating this information would refine the estimation of incidence and disease impacts.

Seroconversion of *Brucella* spp. was significantly associated with elevated milk SCC, supporting our hypothesis that *Brucella* spp. infection induces mammary gland inflammation with compromised milk quality. The underlying pathology involves multifocal interstitial mastitis with interstitial accumulation of macrophages and intra-acinar infiltration of neutrophils (Neta et al., 2010; Xavier et al., 2009), wherein *Brucella* spp. is excreted in substantial quantities into milk during the acute infection phase (Capparelli et al., 2009). We also found that SCC increased with lactation and days in milking for exposed cows (Figure 5.2). This observation might be related to the natural increase of SCC with DIM (Hand et al., 2012) and the pathophysiology of bovine brucellosis (Neta et al., 2010). The notable increase in SCC in seroconverted and consistently positive cows implicates a deterioration of mammary gland health and may result in lower milk quality and reduced milk prices (Hadrich et al., 2018). An SCC ≥ 100,000 cells/mL has been demonstrated to cause milk yield and value losses of 1.20 US$ – 2.06 US$ per cow per day in US dairy herds (Hadrich et al., 2018). Although the Chinese National Food Safety Standard for raw milk (GB 19301-2010) has no requirement for milk SCC (National Health Commission of China, 2010), dairy processing enterprises may impose fines or refuse to purchase milk with high SCC for tank milk samples. It should be emphasized that this penalty for high SCC varies among dairy processing enterprises, and SCC is also measured in tank milk samples rather than individual milk samples. Further investigations into the association between the animal-level prevalence and tank milk SCC could provide deep insights into the effects of brucellosis on tank milk quality and price.

Abortion is one of the most tangible consequences of *Brucella* spp. infection in dairy cows. Many cross-sectional surveys also identified a positive association between *Brucella* spp. seropositivity and pregnancy loss (Deb Nath et al., 2023; Etefa et al., 2022; Zeng et al., 2017), with odds ratios varying from 3.3 and 9.0. Our estimation of relative risk for *Brucella* seroconversion status (RR = 4.26, 95% CI: 3.17, 5.73) also fell within this range. The risk of abortion for consistently negative cows increased with lactation, possibly related to the physiological nature of cows and higher exposure probability to other endemic diseases (e.g., bovine viral diarrhea virus) (Deng et al., 2015). Some studies have also found similar effects of age (lactation) on the risk of abortion (Keshavarzi et al., 2017; Rafati et al., 2010). Seroconverted cows with lactation 4th and more displayed the highest risk of abortion among all groups (Figure 5.3 Panel B). This observation is likely due to cumulative exposure to *Brucella* spp., aging-related physiological stress, and repeated reproductive cycles, exacerbating the impact of infection on pregnancy outcomes. The higher relative risk of abortion in seroconverted cows (RR = 4.26, 95% CI: 3.17, 5.73) compared to consistently positive cows (RR = 3.25, 95% CI: 2.39, 4.42) suggests that recent *Brucella* infection exerts a more severe impact on pregnancy than chronic infection. Acute-phase seroconversion likely triggers a stronger inflammatory response, placentitis, and bacterial invasion of fetal tissues, leading to a higher likelihood of abortion (Neta et al., 2010; Xavier et al., 2009). In contrast, consistently positive cows may have developed partial immune tolerance or lower bacterial load over time, reducing, but not eliminating, abortion risk (The Center for Food Security and Public Health, 2018). This finding underscores the importance of early detection and rapid intervention, as newly infected cows pose the greatest reproductive risk. For dairy herds known to be infected by brucellosis, vaccines should be used to mitigate the effects of brucellosis on production (Blasco et al., 2021; de Oliveira et al., 2022). Longitudinal monitoring of seroconversion allows for a precise understanding of changing disease status and even the time of infection if the test interval is sufficiently short, which enables a more accurate inference of casual relationships with changes in productivity.

Accurately determining the timing of *Brucella* infection is challenging, particularly given the one-year interval between tests in this study. However, infection timing significantly influences production outcomes in dairy cows. Young heifers exposed before mating often remain asymptomatic until their first calving or aborting when clinical manifestations and production losses become apparent (Bercovich, 1998). Alternatively, early embryonic loss or failure to conceive may occur, reducing reproductive efficiency and prolonging calving intervals. Heifers exposed between mating and first calving (or aborting) face a high risk of pregnancy loss as *Brucella* spp. preferentially localizes in the reproductive tract, resulting in placentitis and immediate production impacts (Neta et al., 2010; Xavier et al., 2009). Conversely, cows infected with *Brucella* after the first calving or aborting may experience delayed and chronic effects on fertility and milk production (Bercovich, 1998). This suggests that the impact of infection timing on production warrants further investigation, with more frequent testing needed to clarify how specific timing exposure influences production in dairy cows.

The primary intervention measures against *Brucella* spp. in Chinese dairy herds are test-and-culling, vaccination, and farm biosecurity measures (Zhang et al., 2018). Mellado et al. (2015) demonstrated the positive effect of the vaccination against brucellosis on milk production and abortion based on field observation studies. Holt et al. (2023) identified that test-and-removal is not acceptable and feasible at the early stages of control programs where seroprevalence is high and many animals would be culled due to false positive results. In addition, Dhand et al. (2021) pointed out that the acceptability and feasibility of mitigation measures among farmers should be considered before implementation; otherwise, it will directly affect compliance with recommended policies. Mitigation measures should be carefully studied, considering farmers' interests and available resources before being applied to dairy farms.

This longitudinal study recorded DMY, SCC, and abortion as key outcomes. The Henan DHI has been encouraging dairy farms to use integrated automatic milking and recording systems, which capture DMY with unique identification and minimize observer bias and transcription errors. SCC was measured using a Foss Somatic Cell Analyzer (Fossomatic 5000, Denmark Foss Company) with regular calibration to maintain accuracy. The measures of DMY and SCC were objectively measured and therefore unlikely to be biased. However, abortion events relied on manual reporting by veterinarians and farmers, introducing potential reporting bias and misclassification. Future studies should cross-validate abortion records using multiple data sources, such as farm logs and reproductive parameters (e.g., calving intervals). Additionally, excluding outliers (SCC > 2000 thousand cells/mL or DIM > 400) from data analysis mitigated the influence of outliers, but may underestimate disease effects.

Despite a 6.4% follow-up loss, potential biases were considered by investigating the specific causes of follow-up losses. Potential premature cullings arising from reproductive failure, mastitis, and low milk production may not be randomly distributed relative to routine cullings as the seroprevalence of brucellosis in the follow-up loss population was 2.3 (95% CI: 1.6, 3.3) times higher than that in the retained group. Cattle exposed to *Brucella* are likely at a higher risk of developing these signs and being prematurely culled than unexposed cattle. Consequently, the estimates of incidence and its effects on productivity may be underestimated due to the lack of data from this follow-up loss population. These losses were also considered in our study design by investigating their causes for follow-up loss (See Table S 4.2 in Appendix 4). Although the proportions of reasons for culling are comparable to those previously reported in the general Chinese Holstein cows (Wu et al., 2012), cautions are warranted regarding the groups of animals present on the farm but not captured in the second round of testing and low milk production in the follow-up loss population, given their notable high seroprevalences (44.4% and 40.0%) at the initial test. The lack of such data may underestimate the disease impacts on production. The overall test sensitivity (65.1%) likely resulted in misclassification of infected animals as uninfected, particularly those that seroconverted between tests, leading to an underestimation of true incidence and attenuation of associations with production losses. Furthermore, we acknowledge that other infectious or non-infectious causes may also contribute to abortion, decreased milk yield, and increased SCC, as these productivity outcomes result from multifactorial aetiologies in dairy cattle, such as infections with bovine viral diarrhea virus (Deng et al., 2015), *Mycoplasma bovis* (Timonen et al., 2017), and

Mycobacterium bovis (Mellado et al., 2015), or non-infectious causes such as heat stress and trace element deficiencies in feed (Van Emon et al., 2020). However, it is challenging to investigate multiple pathogens, husbandry, environmental, and nutritional factors within one study. A severe flood in Henan Province in July 2021 may also have affected disease transmission and dairy cow productivity, but its impacts were difficult to estimate. Incorporating data on these factors in future studies would enhance the robustness of our findings.

5.6 Conclusions

In conclusion, *Brucella* spp. significantly affects dairy cattle productivity, as evidenced by reduced DMY, elevated SCC, and increased abortion risk. The greatest production losses were observed in seroconverted cows compared to consistently negative cows, while consistently positive cows also experienced adverse effects, albeit to a lesser extent. These findings suggest that *Brucella* spp. induces substantial production losses during acute infection, with persistent impacts in chronic cases. This highlights the critical need for adopting active countermeasures for positive herds to mitigate brucellosis-related production losses in Henan Province. Future research could explore the long-term financial costs and benefits of different brucellosis control programs.

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In which chapter is the manuscript/published work?	Chapter 6

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Yu Wang: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. Emilie Vallée: Methodology, Supervision, Writing – review & editing. Cord Heuer: Methodology, Supervision, Writing – review & editing. Aizhen Guo: Funding acquisition, Supervision, Writing – review & editing. Youming Wang: Methodology, Supervision, Writing – review & editing. Zhen Zhang: Resources, Supervision, Writing – review & editing. Chris Compton: Conceptualization, Methodology, Supervision, Writing – review & editing.

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Chapter 6 Financial analysis of intervention strategies to control *Brucella* spp. in Henan dairy herds

Yu Wang[a], Emilie Vallée[a], Cord Heuer[a], Aizhen Guo[b], Youming Wang[c], Zhen Zhang[d], Chris Compton[a]

[a] EpiCentre, School of Veterinary Science, Massey University, Private Bag 11-222, Palmerston North, New Zealand

[b] National Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China

[c] China Animal Health and Epidemiology Center, Qingdao, China

[d] Henan Dairy Herd Improvement Center, Zhengzhou, Henan, 450045, China

Preface

Chapter 4 found that dairy farmers were motivated to adopt interventions by the impact of disease on production and economic returns, and Chapter 5 further confirmed the negative effects of brucellosis on milk production, pregnancy, and somatic cell count. Economic considerations are critical in designing and implementing an effective brucellosis control program in China and elsewhere (Singh et al., 2018). Farm stakeholders face complex decisions when choosing between vaccination and test-and-culling strategies, as these interventions require careful evaluation of long-term financial costs and benefits. However, the financial analysis of these control measures in Chinese dairy herds has never been assessed.

Chapter 6 addresses this gap by conducting a financial analysis of brucellosis control strategies at the herd level. The baseline scenario assessed the financial losses caused by brucellosis in the absence of interventions, while three additional scenarios evaluated the economic value of (1) vaccination, (2) test-and-culling, (3) test-and-culling plus vaccination by calculating the costs and benefits of each program compared to the baseline scenario. This study considered the animal health impacts of the vaccination or test-and-culling programs with/without government compensation from the perspective of farmers. The findings provide essential financial evidence to support farm stakeholders in selecting the most financially beneficial intervention measures.

6.1 Abstract

Bovine brucellosis, primarily caused by *Brucella abortus*, presents significant economic challenges in the dairy sector in China, where economic studies are scarce. This study aims to: (1) quantify the financial impact of brucellosis in Chinese dairy herds at the herd level and (2) evaluate the benefit-cost outcomes of the following intervention approaches: (a) annual test-and-culling of adult cows; (b) vaccination for the entire herd at the initial year followed by annual vaccination of replacement heifers; and (c) test-and-culling at the initial year and annual vaccination of replacement heifers. Using longitudinal data for estimating disease impacts and financial information, we projected the costs and benefits of each scenario at the herd level over ten years, followed by a cost-benefit analysis. Based on a typical herd size of 600 animals, the study estimated the annual costs of brucellosis to be 47.4 thousand Chinese Yuan (CNY, 95% CI: 34.5 – 60.7), with annual expenses per cow of 78.9 CNY (95% CI: 57.2 – 101.1). Decreased milk production accounted for about 70% of total costs. The cost-benefit analysis revealed that all intervention scenarios yielded positive Net Present Values (NPVs) and Benefit-Cost Ratios (BCRs) above one, with vaccination being the most cost-effective strategy offering the highest BCR of 5.84 (95% CI: 4.34 – 7.42) and NPV of 408.2 thousand CNY (95% CI: 281.9 – 541.2 thousand CNY) over ten years, followed by a combination of 1st year test-and-culling and vaccination (BCR: 4.23, 95% CI: 3.19 – 5.31), and test-and-culling alone (BCR: 2.43, 95% CI: 1.87 – 3.00). Sensitivity analysis confirmed the robustness of the main findings across various parameters. The study suggests vaccination is the most economically favorable intervention to mitigate economic losses from brucellosis in dairy farms. Adopting vaccination programs would substantially reduce disease prevalence and enhance economic outcomes in the Chinese dairy sector. These estimates have significant implications for dairy farmers in understanding the economic rationale for developing and implementing cost-effective measures for controlling brucellosis in dairy cows in Henan Province and, more broadly, probably in other provinces in China.

Keyword: Economic impact; *Brucella* spp.; Dairy cattle; Cost-benefit analysis; Vaccination; Test-and-culling

6.2 Introduction

Brucella abortus (*B. abortus*) is the primary cause of bovine brucellosis in dairy cattle globally, resulting in significant reproductive and production loss due to abortion, reduced milk yield, infertility, arthritis, and placental retention (Godfroid et al., 2011; Seleem et al., 2010; Zhang et al., 2018). This zoonosis also poses essential risks to human health through direct or indirect contact with animals or contaminated animal products (e.g., fetus and placenta), consumption of raw milk and undercooked meat from infected animals, and inhalation of contaminated aerosols (Pappas, 2022). Consequently, brucellosis has resulted in considerable economic losses and health burdens to the dairy industry and public health worldwide, including in China and most developing countries (Laine et al., 2023; Peng et al., 2020b). Successful animal brucellosis elimination programs have involved mass vaccination, test-and-culling, improved farm biosecurity, animal movement management, continuous disease surveillance, enhanced disease awareness, and adequate funding (Zhang et al., 2018). However, implementing these interventions is complex and requires alignment with specific livestock production practices, financial circumstances, and justification of financial benefits and costs of candidate control programs (McDermott et al., 2013).

Brucellosis remains endemic in dairy cattle and humans in most provinces of China (Lai et al., 2017; Ran et al., 2018; Ran et al., 2019; Wang et al., 2024b; Wang et al., 2021b). The animal-level prevalence in dairy cattle (Ran et al., 2019) and incidence in humans (Lai et al., 2017; Wang et al., 2021b) have been increasing, constituting severe concerns for the Chinese dairy industry and public health. Therefore, brucellosis has been listed as a priority animal disease for control in China (Chen et al., 2021). In 2017, brucellosis in human populations resulted in an economic loss of 31.29 million US dollars in China (Peng et al., 2020b). To address this challenge, China has launched a series of policies to control the disease since March 2022 (Ministry of Agriculture and Rural Affairs, 2022), including (1) a disease surveillance program in dairy cattle populations; (2) a vaccination program; (3) a test-and-culling program; (4) accreditation of brucellosis-free herds or regions; (5) animal movement management; and (6) improvements in farm biosecurity. Despite these efforts, the efficacy of these measures heavily depends on government funding and farmer engagement, which are highly influenced by the perceived economic gains from implementing such measures (Zeng et al., 2019a). Therefore, it is essential to inform farmers about the direct losses attributable to brucellosis and the financial rationale behind the control programs to enhance their compliance with brucellosis control programs.

Dairy herd decision-makers face challenges in selecting the optimal control strategy to achieve the maximum financial return at minimum cost, especially between mass vaccination or test-and-culling strategies. Recent economic evaluations suggest that vaccination is most cost-effective compared to the test-and-culling strategy; however, the financial assessment may differ considerably given variable epidemiological, agricultural, and socioeconomic conditions such as disease prevalence and financial value for animal products (Kiiza et al., 2023; Singh et al., 2018; Zeng et al., 2019b). To this end, we have conducted a longitudinal study (Chapter 5) to estimate the effect of *Brucella* spp. on milk production and pregnancy outcomes in twelve Henan dairy herds. These empirical data can serve as a population-specific benchmark to improve the accuracy of our estimations in this study. Henan Province, China, held about 407,300 dairy cattle and produced 2.1 million tons of dairy products in 2022, making it one of the significant dairy-producing provinces in China (National Bureau of Statistics of China, 2023). It is therefore necessary to justify the financial benefits of the candidate control schemes.

To date, no published studies have reported the direct losses attributable to brucellosis and the cost-benefit outcomes of various control programs on intensively housed dairy herds in China. Using a stochastic cost-benefit analysis model and observational data from a longitudinal study on the Henan dairy herd, our study aimed to: (1) estimate the direct losses attributable to brucellosis and (2) evaluate the cost-benefit outcomes of three brucellosis control strategies.

6.3 Materials and Methods

6.3.1 The study area and design

Henan Province, located in central China, is one of the major agricultural and dairy milk production provinces in China. A previous seroprevalence survey among non-vaccinated dairy herds in Henan Province in 2018 indicated that brucellosis was endemic, with an apparent prevalence of 0.12%-3.69% at the animal level and 2.58%-24.24% at the herd level across five regions (Zhao et al., 2020). However, there are no published reports providing information about the prevailing *Brucella* species in both dairy cattle and humans in Henan Province (Wang et al., 2024b), and we assume that *B. abortus* is the primary causative agent for bovine brucellosis in Henan dairy herds. Despite various nationwide control measures, the incidence of human brucellosis and prevalence in dairy cattle have risen significantly over the past decade

(Ran et al., 2019; Wang et al., 2021b), highlighting potential gaps in the adoption and maintenance of control measures. Public data from the National Veterinary Bulletin reported only six new cases of brucellosis in cattle in Henan in 2021, all of which were subject to compulsory culling (Ministry of Agriculture and Rural Affairs, 2022). However, the number of cases related to cows or herds was not stated in the National Veterinary Bulletin. We optimistically assume previous control measures have had limited effects on brucellosis control and are not considered in the following analysis. Vaccination of dairy cattle with *Brucella* vaccines was previously discouraged due to its interference with serological tests. However, with the release of the new five-year action plan (Ministry of Agriculture and Rural Affairs, 2022), vaccination has become an alternative intervention with approval from the county veterinary departments.

One baseline and three intervention scenarios for brucellosis control were investigated, primarily based on published literature and recently released government policies (Ministry of Agriculture and Rural Affairs, 2022). Scenario 1 was the absence of specific intervention measures (baseline model); Scenario 2, annual test-and-culling; Scenario 3, mass vaccination for all animals at the 1st year and annual vaccination for replacement calves and heifers; and Scenario 4, test-and-culling at the 1st year and annual vaccination for replacement calves and heifers (Table 6.1). The design of this study was based on the perspective of dairy farmers, and government costs in disease control were not considered in this study. Dairy farmers will be responsible for the expenses of all control measures, while government incentives (e.g., compensation for culled animals and vaccine procurement) derived from adopting intervention measures were considered one of the farmers' benefits.

Table 6. 1 Four intervention scenarios and implementation times used in this cost-benefit analysis of brucellosis in one typical Henan dairy herd

Scenario	Specification	Years for vaccination	Years for test-and-culling
1 (Baseline)	Without vaccination or test-and-culling	Not applicable	Not applicable
2	Annual test-and-culling	Not applicable	1 to 10
3	Vaccination for whole herd at 1st year and annual vaccination for replacement	1 to 10	Not applicable
4	Test-and-culling at 1st year and annual vaccination replacements thereafter	2 to 10	1

6.3.2 Financial analysis

6.3.2.1 Data Sources and Model Input Parameters

Input parameters for the financial analysis were derived from field surveys (Chapters 4 and 5) or published literature, where no data was available in the study area (National Bureau of Statistics of China, 2023). A typical Henan dairy herd for our model consisted of a median herd size of 600 animals (interquartile range: 300, 2000), including about 60% adult cows (over two years of age) and 40% replacement young females (less than two years of age).

A stochastic cost-benefit analysis model was developed using a Microsoft Excel spreadsheet and an add-in software @RISK version 7.6 (Palisade Corporation, New York, USA). Cost-benefit analysis (also called benefit-cost analysis) is a helpful approach for comparing the financial benefits of alternative disease control programs at national, regional, or herd levels over an extended horizon period, typically five to twenty years (Marsh, 1999) while accounting for differing value of money over time. A beta distribution was used in this assessment to account for uncertainty in the prevalence of disease in Henan dairy populations. Only adult females were considered in the model of direct costs of disease effects since limited effects occurred in replacements before their first calving or abortion. All replacement calves were assumed to be vaccinated once with a standard dose of *B. abortus* A19 strain when they reached six months of age in Scenarios 3 and 4. A Pert distribution was used with data from the literature or public data to specify the minimum, mode (most likely), and maximum values of the market price for milk and meat. The model inputs and parameters are presented in Table 6.2.

Table 6. 2 Input parameters used in the cost-benefit analysis model of brucellosis in Henan dairy herds

Parameters	Description	Value	Reference/source
Herd basic characteristics			
Herd size	Number of all-aged animals in a typical dairy herd	600	Chapter 4
Proportion of adult cows	Proportion of adult cows within herd	60%	Assumption
Fertility rate per year (%)	Average number of calving per animal year, calculated from the average calving interval (405 days)	90%	Henan DHI (2023)
Average 305-day milk yield (kg per cow)	Average 305-day milk yield	9700.8	Henan DHI (2023)
Average body weight for adult cow (kg per cow)		Normal distribution (605.4, 68.1)	Xie et al. (2022)
Disease impacts on productivity			
Apparent prevalence of *Brucella* spp. infection	Assume a median apparent prevalence of 2.1%	2.1%	Wang et al. (2024b)
Reduction in milk yield of infected cows over one year	Most likely value with minimum and maximum	Pert distribution (0.10, 0.046, 0.153)	Chapter 5
Probability of infected cows aborting over one	Most likely value with minimum and	Pert distribution (0.235,	Chapter 5

Parameters	**Description**	**Value**	**Reference/source**
year	maximum	0.153, 0.318)	
Meat loss for infected cows (%)	Percentage of relative reduction	5%	Bernués et al. (1997)
Mortality (%)	Mortality of cows that aborted and tested positive	1%	Bernués et al. (1997)
Perinatal mortality (%)	Of calves born to infected dams	10%	Bernués et al. (1997)
Replacement cost per animal (CNY)	Difference between value of a replacement heifer and the residual value of a culled cow from meat	Calculation	Hugh-Jones et al. (1975)
Increased replacement risk	Average increase in the risk of replacement of test-positive cows	15%	Bernués et al. (1997)
Price information			
Carcass price (CNY/kg)	Most likely value with minimum and maximum carcass price in 2019-2024	Pert distribution (14, 9, 16)	Department of Agriculture and Rural Affairs of Henan Province (2022c)
Average price for a calve in 2022	CNY	2,000	Personal communication (Zhijie Xiang)
Average price for a replacement heifer in 2022	CNY	11,000	Personal communication (Zhijie Xiang)
Average price for an adult	CNY	18,000	Personal communication (Zhijie

Parameters	Description	Value	Reference/source
cow in 2022			Xiang)
Compensation for a culled cow	CNY	6,000	(Department of Agriculture and Rural Affairs of Henan Province, 2022b)
Loss from an abortion (CNY)	Assume the same value as a calf	2000	Personal communication (Zhijie Xiang)
Cost for RBT testing	CNY per sample	20	Personal communication (Zhijie Xiang)
Cost for SAT testing	CNY per sample	30	Personal communication (Zhijie Xiang)
Parameters for disease control programs			
Proportion of sampling	Proportion of sampled cows for test-and-culling	50%	Assumption
Combined sensitivity (%) in series	RBT & SAT interpreted in series	61.2%	Wang et al. (2024a)
Combined specificity (%) in series	RBT & SAT interpreted in series	99.9%	Wang et al. (2024a)
Combined sensitivity (%) in parallel	RBT & SAT interpreted in parallel	88.5%	Wang et al. (2024a)
Combined specificity (%) in parallel	RBT & SAT interpreted in parallel	99.2%	Wang et al. (2024a)

Parameters	**Description**	**Value**	**Reference/source**
Consumables cost for vaccine administration or blood collection	CNY per animal	5	Personal communication (Zhijie Xiang)
Brucella abortus A19 vaccine	CNY per standard dose	20	Henan Government Procurement Centre (2022)
Average number of samples post-vaccination in a dairy herd	At least 20 samples per herd, we assumed an average of 30	30	Department of Agriculture and Rural Affairs of Henan Province (2022b)
Vaccine efficacy (%), the percentage of vaccinated cattle that are protected against abortion caused by *Brucella* spp.	95% confident that vaccine efficacy is > 0.481 with a mode of 0.751	Beta distribution (8.43, 3.46)	de Oliveira et al. (2022)

Our longitudinal study (Chapter 5) suggests that brucellosis could result in milk loss (Range: 4.6%-15.3%, at a mode of 10.0%) and abortions (23.5%, 95% CI: 15.3, 31.8) in seropositive cows. Other direct disease impacts (e.g., perinatal mortality, replacement) are elaborated in Table 6.2 (Bernués et al., 1997; Hugh-Jones et al., 1975). The price values for milk, meat, calves, heifers, cows, and vaccines used in this assessment are shown in Table 6.2 (Department of Agriculture and Rural Affairs of Henan Province, 2022c; Henan Government Procurement Centre, 2022). The cost of testing depends on the testing strategy. In serial testing, the cost of SAT depends on the RBT test result (SAT cost per sample = $Cost_{SAT}*[Se_{RBT}*TP+(1-Sp_{RBT})*(1-TP)]$) , where TP represents true prevalence, derived from apparent prevalence and sensitivity and specificity using Rogan-Gladen formula (Rogan et al., 1978). However, in parallel testing, RBT and SAT costs depend only on the number of tests and the unit price, regardless of the test results. We assumed that the average loss per abortion was the same as the value of one calf since indirect side effects (e.g., reduction in milk yield) associated with abortion were separately accounted for. The currency of the monetary values used in this study is the Chinese Yuan (CNY), with 1 CNY assumed to equal 0.15 US Dollars (USD) in 2022 (The World Bank, 2022).

To simplify the model, we made several assumptions: (1) the demographic structure within the herd remained stable; (2) animals newly infected remained infected lifelong; (3) the number of female calves raised every year for replacement purposes equaled the number of cows that died or were culled; (4) brucellosis prevalence was at endemic equilibrium without any interventions. Therefore, the number of animals infected by an infected animal during the infectious period was equal to one ($R_e = 1$), and $R_e = R_0*S^*$, where R_0 is the basic reproduction number that indicates the number of newly infected animals produced by an infected animal over an entire infectious period in a disease-free population, S^* is the proportion of susceptible adult cows in the total population of animals (Hegazy et al., 2009). Given the chronic nature of *Brucella* spp. infection (Neta et al., 2010), we assumed that replacement calves and heifers are not susceptible or affected by the disease until they give birth or have a late abortion. Under these assumptions, the number of newly infected animals each year should equal the number of infected animals being removed from the population.

The initial true prevalence was calculated by the apparent prevalence with diagnostic sensitivity and specificity using the Rogan-Gladen adjustment formula (Rogan et al., 1978). In Scenario 2, the true prevalence of brucellosis at year n ($TPrev_n$) was calculated as follows: $TPrev_n = TPrev_{n-1} * (1 - F) + TPrev_{n-1} * F * DSe$, where the true prevalence ($TPrev_{n-1}$) is the true prevalence in the previous year, F is the fraction of the population for test-and-culling annually, and DSe is the overall diagnostic sensitivity.

In Scenario 3, the true prevalence of brucellosis at the year n ($TPrev_n$) is calculated as follows: $TPrev_n = TPrev_{n-1} * \left(1 - V_c * (1 - Prob_f\right) * VE$, where V_c is the vaccination coverage in a dairy herd, $Prob_f$ is the probability of failure in vaccine administration, and VE is the vaccine efficacy.

In Scenario 4, the true prevalence of brucellosis at the year n is calculated as described above, and the only difference is that test-and-culling is performed in the first year, followed by vaccination in the years (second to tenth).

6.3.2.2 Financial components of brucellosis and interventions

A cost-benefit analysis framework was adapted from that reported by Rushton et al. (1999), incorporating government compensation for culled animals and vaccine procurement as benefits for farmers complying with government policy (Figure 6.1). Table 6.3 details the specific benefit and cost components and their calculation approaches. Reduced disease loss components include milk production, meat production, pregnancy, mortality, perinatal mortality, and increased replacement (Alves et al., 2015; Bernués et al., 1997; Hugh-Jones et al., 1975). Data on other consequences (e.g., orchitis, mastitis, and increased conception failure) were not included as there was no available data regarding these signs of the disease in dairy cattle.

The costs associated with the brucellosis intervention programs were calculated separately according to the respective interventions, as shown in Figure 6.1 and Table 6.3. Data on the government expenditures on vaccine procurement in Henan were available on the public website of Henan Government Procurement Centre (Henan Government Procurement Centre, 2022). Under the current policy, dairy farmers purchase the vaccine themselves in advance, and then local governments compensate for vaccine procurement based on the government vaccine procurement price (Department of Agriculture and Rural Affairs of Henan Province, 2022b). After consulting with three vaccine retailers, we assumed that the purchase price for farmers was the same as the government procurement price. Government vaccine compensation was considered to be a benefit derived from the adoption of vaccination intervention rather than a reduction in intervention costs. The expenditure on post-vaccination monitoring was estimated from the serology test costs undertaken in the vaccination years in Scenarios 3 and 4. The expenses of serological tests were determined by multiplying the total number of samples to be tested by the price per sample. According to Henan government regulations, a vaccinated dairy herd needed to collect at least twenty samples for post-vaccination monitoring, so we optimistically assumed that each vaccinated herd would collect an average of thirty samples per year (Department of Agriculture and Rural Affairs of Henan Province, 2022b). The cost of a single sample was equal to the sum of the consumables fee for sample collection, and RBT and SAT costs. We assumed that one veterinarian could collect sixty blood samples or vaccinate sixty animals per day with a daily wage of 300 CNY (National Bureau of Statistics of China, 2023). The number of veterinarians depends on the availability of each farm. For simplicity, we used a labor cost of 5 CNY per animal for blood collection or vaccination administration.

The cost of the test-and-culling intervention (Scenario 2) was determined by calculating two components: the testing budget and the culling-and-replacement budget. The testing budget was calculated as the cost of testing per sample multiplied by the number of samples tested. The costs for culling and replacement only included the value of the replacement heifers because the government pays for the slaughter cost for seropositive cows. According to the government regulations (Ministry of Agriculture and Rural Affairs, 2022), the compensation for each culled seropositive cow was 6,000 CNY per head, which was regarded as a benefit of adopting test-and-culling intervention for farmers.

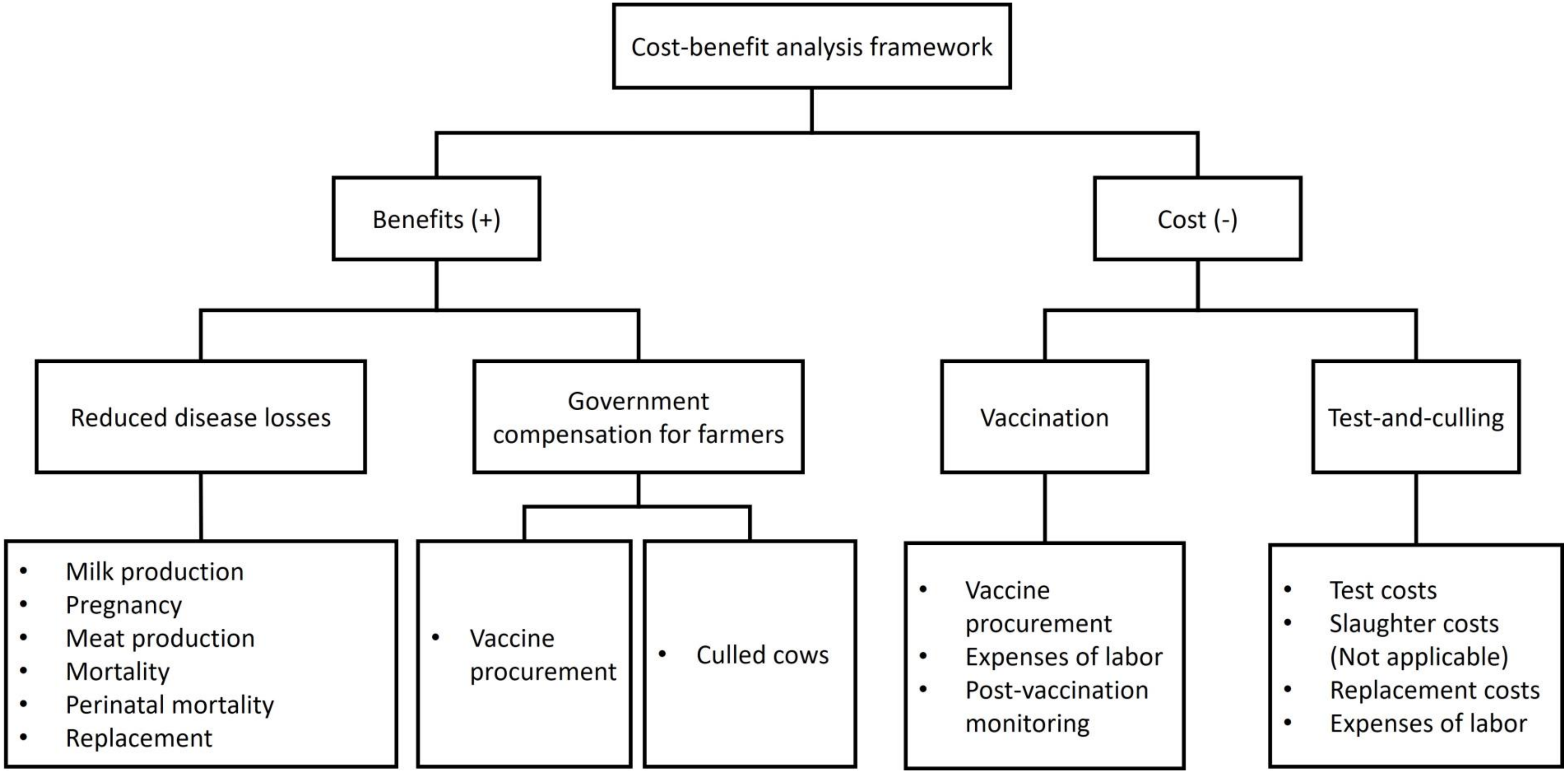

Figure 6. 1 Cost-benefit analysis framework for evaluating financial impacts of disease and benefits and costs of three interventions, compared to the baseline scenario (lack of strict interventions) in a dairy herd in Henan Province, China.

Table 6. 3 The benefit and cost components and their calculation approaches in the cost-benefit analysis.

Category	Component	Calculation approach
Benefits	**Reduced disease expenditure**	Reduced disease expenditure = Milk production + Meat production + Pregnancy + Mortality + Perinatal mortality + Increased replacement
	Milk production	Milk = Number of positive cows × Pregnancy rate × 305-day milk yield × Reduction in milk yield in infected cows × Milk price
	Meat production	Meat production = Number of positive cows × Average weight of a cow × Reduction in meat production × Meat price
	Pregnancy	Pregnancy = Number of positive cows × Pregnancy rate × Risk of abortion × Average losses per abortion
	Mortality of aborted cows	Mortality loss = Number of positive cows × Pregnancy rate × Risk of abortion attributable to *Brucella* spp. infection in pregnant cows × Mortality × Average price of a cow
	Perinatal mortality of calves born from infected dams	Perinatal mortality = Number of positive cows × Pregnancy rate × Perinatal mortality × Value of a calf
	Increased replacement of test-positive cows	Increased replacement = Number of positive animals × Increase in replacement requirements × Replacement cost per animal
	Government compensation	
	Vaccine procurement	Vaccine = Number of animals being vaccinated × Price of vaccine per standard dose
	Culled cows	Culled cows = Number of test-positive animals × Compensation value per culled cow
Costs	**Test-culling-replacement**	Test-culling-replacement = Test costs + Slaughter costs + Replacement

Category	Component	Calculation approach
	Test costs	Test costs = Number of samples × Testing cost per sample
	Slaughter costs	Not applicable as the government affords this cost in China
	Replacement	Replacement = Number of culled cows × Value of a replacement heifer
	Expense of labor	Expense of labor = Number of vaccinator × Number of working day × Average salary per day
	Vaccination	Vaccination costs = Vaccine procurement + Expense of labor + Post-vaccination monitoring
	Vaccine procurement	Vaccine = Number of animals being vaccinated × Price of vaccine per standard dose
	Expense of labor	Expense of labor = Number of animals being sampled or vaccinated × Average labor cost per animal
	Post-vaccination monitoring	Post-vaccination cost = Number of samples × Testing cost per sample
	Combination of test-and-culling and vaccination	Costs = Test-culling-replacement costs + Vaccination costs

6.3.2.3 Cost-benefit analysis

An average discount rate (r) of 4.8% between 1987 and 2022 was applied to adjust the sum of future currency back to the present sum they represent (The World Bank, 2023). Initially, we calculated the annual costs attributable to disease in the baseline model (Scenario 1). The yearly costs ($Cost_t$, t = 1,2,…10) and benefits ($Benefit_t$, t = 1,2,…10) from averted animal productivity losses in infected animals for each intervention strategy were estimated to calculate the net present value (NPV) and benefit-cost ratio (BCR) (Dijkhuizen et al., 1995; Marsh, 1999). In Scenario 1, the total cost equaled the expected losses from brucellosis due to the absence of intervention measures. In Scenarios 2, 3, and 4, specific interventions were implemented (Table 6.1), with costs reflecting the required inputs and benefits measured as the prevented production losses. The costs included vaccine procurement, post-vaccination monitoring, testing, and replacement heifers. The averted losses and compensation for vaccine procurement or culled animals were considered benefits for adopting relevant intervention programs for dairy farmers (Montiel et al., 2015; Singh et al., 2018). The losses attributable to brucellosis per infected animal were estimated as described previously. Public health benefits were not considered because our primary focus was the financial analysis of brucellosis in dairy cattle rather than in humans.

The analyses were conducted using an add-in @RISK version 7.6 (Palisade Corporation, New York, USA) in the Microsoft Excel spreadsheet. A Monte Carlo simulation using Latin hypercube sampling with 10,000 iterations was performed to simulate the distribution and to account for the variability and uncertainty associated with relevant variables. The results are presented as a bar plot with a median value and 95% credible intervals (CI) using the ggplot2 package (Wickham, 2016) in R statistical program version 4.1.2.

6.3.3 Sensitivity analysis

A sensitivity analysis was performed in @RISK functions to identify which input parameters had the most effect on the uncertainty of BCR by checking the regression coefficients (R^2) using the built-in standard multiple regression analysis. In addition, we manually changed the parameters involving the apparent prevalence of *Brucella* spp., sampling proportions for test-and-culling, vaccine efficacy, parallel testing, partial or no compensation for culled animals, and vaccine procurement to explore their potential impacts on the BCR and NPV estimation. A total of thirteen supplementary scenarios were investigated, and the specific parameters and details can be found in Table S 5.1 of Appendix 5.

6.4 Results

6.4.1 Economic losses due to *Brucella* spp.

Based on our simplifying assumptions, in a baseline scenario without intervention measures (Scenario 1), a 600-head dairy infected with *Brucella* spp. would maintain a true prevalence of 3.3% over the next ten years. In contrast, the true prevalence was expected to decrease to a very low level (< 0.1%) over ten years under the other three intervention scenarios (Figure 6.2). Without interventions, the average annual economic losses due to *Brucella* spp. were estimated at 47,364 CNY (95% CI: 34,520 – 60,701) per 600-head dairy herd, equivalent to an average loss of 78.9 CNY (95% CI: 57.2 – 101.1) per animal. The average loss for all infected cows in the study was estimated at 4,019 CNY (95% CI: 2,910 – 5,147) per infected cow per year. The most significant contributor to the overall loss was associated with milk yield (33,155 CNY, 95% CI: 20,333 – 46,004), followed by replacement cost (4,162 CNY, 95% CI: 730 – 7,430), pregnancy (4,075 CNY, 95% CI: 3,067 – 5,088), meat (3,909 CNY, 95% CI: 2,818 – 5,052), perinatal mortality (1,733 CNY), and mortality (367 CNY, 95% CI: 276 – 458) (Figure 6.3).

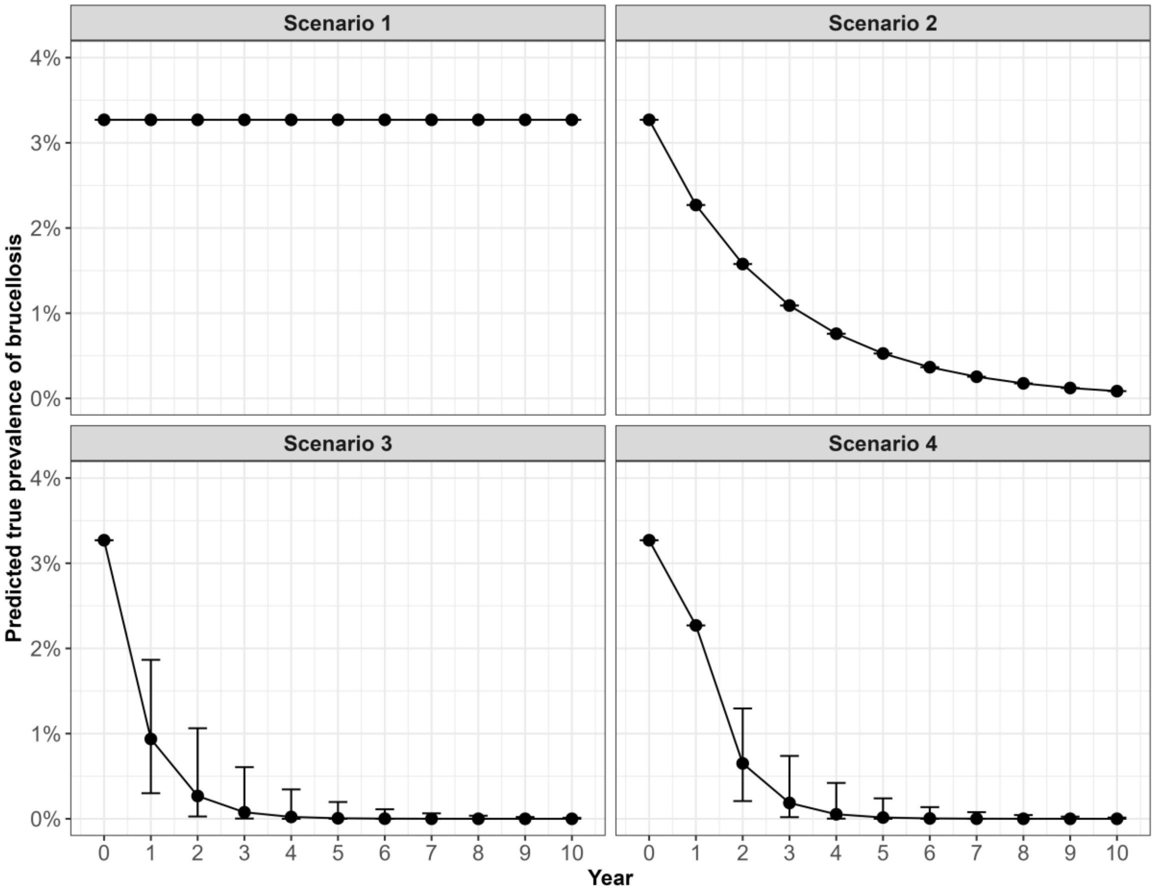

Figure 6. 2 Predicted true prevalence (dot) of brucellosis and its 95% credible interval (error bar) under four scenarios (listed in Table 6.1) over ten years, there are no error bars in Scenarios 1 and 2 because parameters of prevalence and sensitivity and specificity of tests were fixed values.

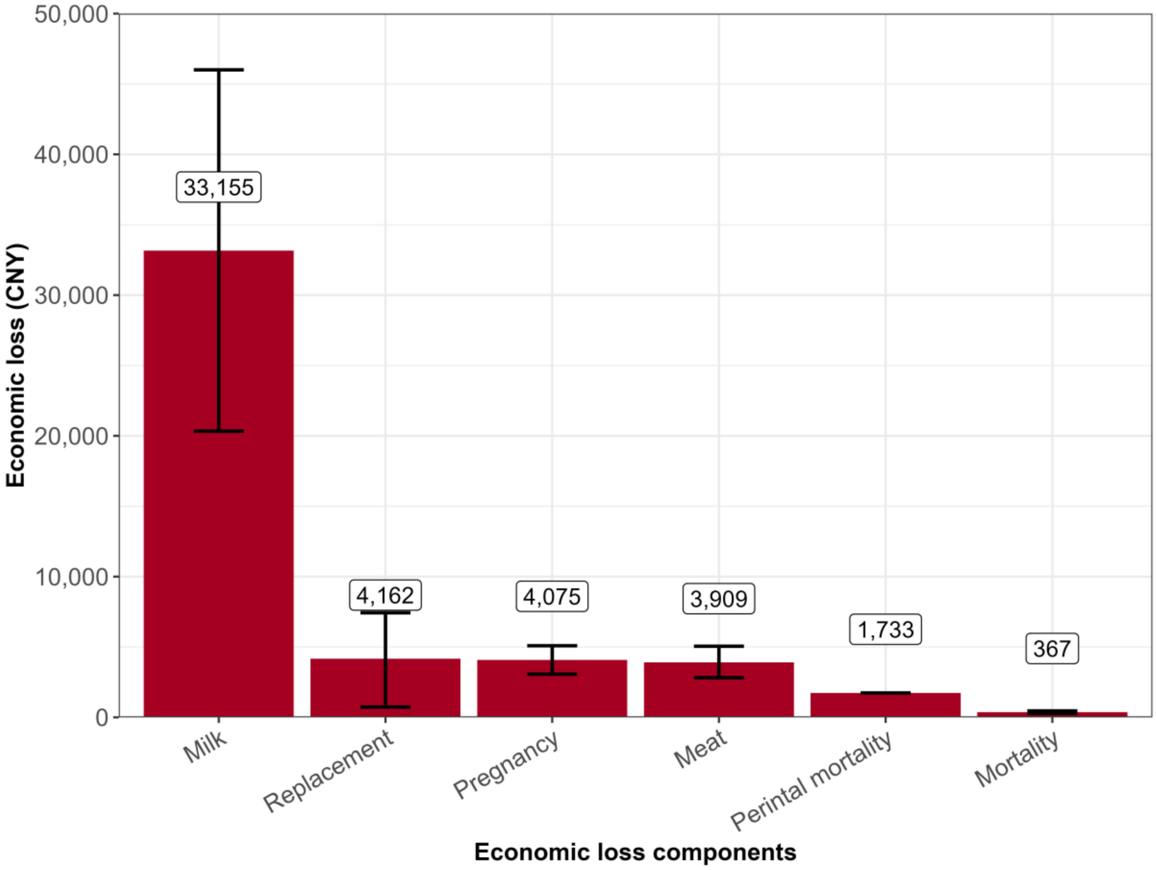

Figure 6. 3 The bar chart shows the estimated average annual economic loss components in median (bar and label) and 95% credible interval (error bar) regarding *Brucella* spp. in a study farm (600 animals).

6.4.2 Cost-benefit analysis of interventions

The financial costs and benefits of these interventions over ten years are presented in Figure 6.4. All three intervention scenarios yielded a BCR greater than one, indicating the financial viability of interventions, with positive NPVs over 200 thousand CNY (Table 6.4). Scenario 3, which involved mass vaccination followed by annual vaccination for replacement, offered the largest BCR (5.84, 95% CI: 4.34 – 7.42) and NPV (408.2 thousand CNY, 95% CI: 281.9 – 541.2) among the three scenarios. Scenario 2 (test-and-culling alone) exhibited the smallest

BCR (2.43, 95% CI: 1.87 – 3.00) and NPV (251.9 thousand CNY, 95% CI: 153.6 – 352.0). The combined approach in Scenario 4 presented the intermediate values for BCR (4.23, 95% CI: 3.19 – 5.31) and NPV (360.8 thousand CNY, 95% CI: 245.0 – 481.0). Scenario 3 consistently showed profitability throughout the predicted ten years, and Scenarios 2 and 4 transitioned from losses to profits by the second year of implementation (Figure 6.4).

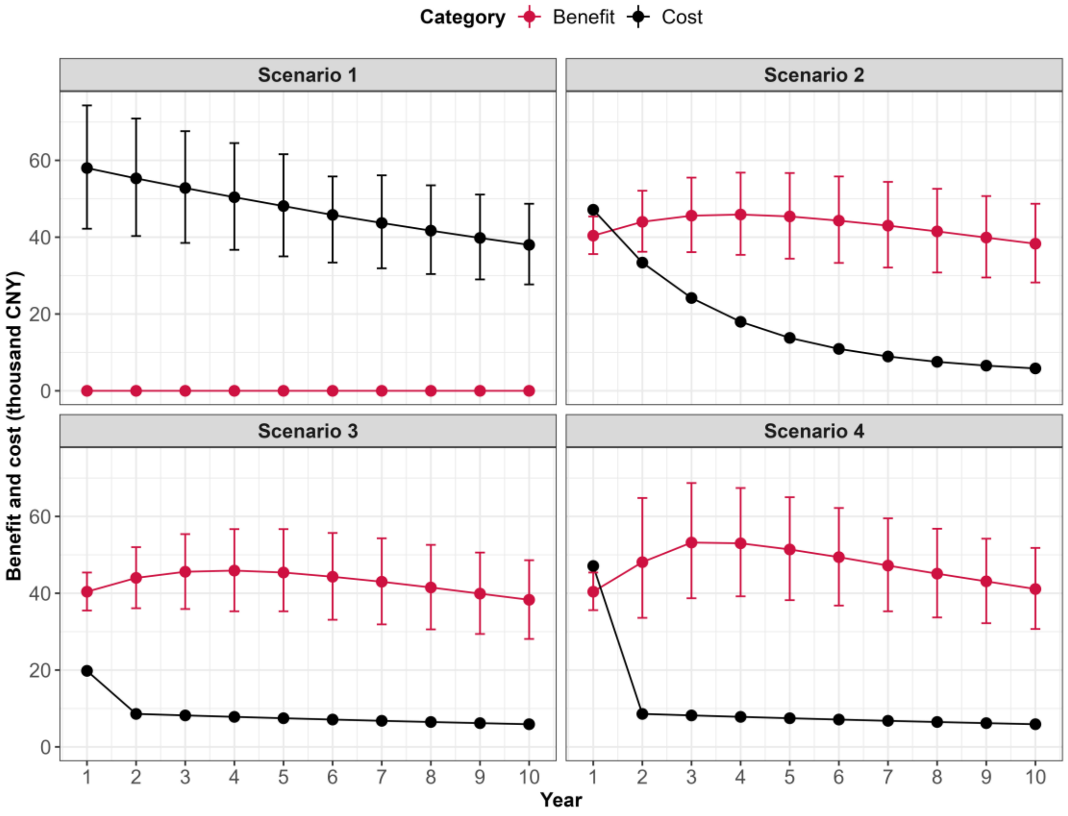

Figure 6. 4 Annual costs and benefits under four scenarios on a 600-head dairy herd in Henan Province, China at an average monetary discount rate (r = 0.048).

Table 6. 4 Cost-benefit analysis for evaluating four scenarios to control brucellosis in a 600-animal dairy herd over ten years in Henan Province

Intervention	Scenario specification	Present value of costs (95% CI)[a]	Present value of benefits (95% CI)[a]	Net present value (95% CI)[a]	Benefit-cost ratio (95% CI)
Scenario 1	No intervention	473.5 (342.9 – 606.5)	0.00	-473.5 (-606.5 – -342.9)	0.00
Scenario 2	Test-and-culling	176.2	428.1 (329.8 – 528.5)	251.9 (153.6 – 352.0)	2.43 (1.87 – 3.00)
Scenario 3	Vaccination	84.3	492.5 (366.2 – 625.6)	408.2 (281.9 – 541.2)	5.84 (4.34 – 7.42)
Scenario 4	Combination[b]	111.7	472.5 (356.7 – 592.7)	360.8 (245.0 – 481.0)	4.23 (3.19 – 5.31)

[a]: expressed in one thousand Chinese Yuan (CNY), [b]: test-and-culling in 1st year and vaccination in 2nd-10th year, CI: credible interval

6.4.3 Sensitivity analysis

Our sensitivity analysis revealed that the initial apparent prevalence significantly influences the BCR of the vaccination program. Increasing the apparent prevalence from 2.1% to 5.0% raised the BCR from 5.84 to 13.52 (95% CI: 9.86 – 17.39), and the NPV increased from 408.2 thousand CNY to 1,062.6 thousand CNY (95% CI: 747.5 – 1,382.1). In contrast, variations in the initial apparent prevalence had a less pronounced effect on the test-and-culling strategy (Scenarios sA and sB, Table 6.5). Adjusting the sampling proportion for test-and-culling (±10%) slightly influenced the BCR and NPV outcomes. The alternative parallel testing strategy (Scenario sE) considerably reduced BCR (1.55, 95% CI: 1.24 – 1.87) and NPV (195.4 thousand CNY, 95% CI: 84.2 – 308.5). Changes in vaccine efficacy assumptions slightly altered BCR and NPV uncertainties without significantly affecting the benefit-cost analysis outcomes (Scenario sF and sG). Scenarios sH-sM demonstrated that all three interventions remained profitable (defined as BCR > 1 and NPV > 0) for farmers over ten years with half or no government compensation for vaccine procurement and culled animals (Table 6.5).

The regression analysis (Figure 6.4) identified that the reduced milk yield due to *Brucella* spp. infection (absolute coefficients > 0.90) had the most significant effect on the cost-benefit results for four Scenarios. The average milk price ($R^2 = 0.19$) ranked as the second most influential factor for Scenarios 1 and 2. For the vaccination and combination programs (Scenarios 3 and 4), the vaccine efficacy ($R^2 = 0.25$ and 0.19) and milk price ($R^2 = 0.19$ and 0.18) had a considerable positive effect on the cost-benefit results.

Table 6. 5 Sensitivity analysis explores the impact of manually changing input variables (refer to Table S 5.1 of Appendix 5 for specific changes) on the benefit-cost ratio (BCR) and net present value (NPV)

Scenarios	Changed variable	Interventions	BCR (95% CI)	NPV (95% CI), thousand CNY (Chinese Yuan)
sA	Lower apparent prevalence	Test-and-culling	1.75 (1.36 – 2.15)	84.6 (41.1 – 129.6)
		Vaccination	2.93 (2.25 – 3.65)	163.2 (105.4 – 223.4)
		Combination	2.59 (2.00 – 3.20)	142.5 (89.5 – 197.2)
sB	Higher apparent prevalence	Test-and-culling	3.00 (2.31 – 3.71)	690.8 (452.6 – 937.7)
		Vaccination	13.52 (9.86 – 17.39)	1,062.6 (747.5 – 1,382.1)
		Combination	6.53 (4.87 – 8.29)	936.9 (655.3 – 1,233.8)
sC	Lower sampling percentage	Test-and-culling	2.46 (1.90 – 3.03)	230.9 (142.4 – 322.1)
		Combination	4.52 (3.41 – 5.70)	360.3 (246.6 – 480.4)
sD	Higher sampling percentage	Test-and-culling	2.37 (1.83 – 2.93)	264.4 (160.0 – 371.7)
		Combination	3.99 (3.01 – 4.99)	361.9 (243.2 – 483.2)
sE	Parallel testing	Test-and-culling	1.55 (1.24 – 1.87)	195.4 (84.2 – 308.5)
		Combination	3.35 (2.56 – 4.16)	352.0 (233.6 – 473.1)
sF	Weakly informative priors for vaccine efficacy	Vaccination	5.79 (4.25 – 7.41)	403.8 (273.9 – 540.9)
		Combination	4.20 (3.15 – 5.31)	357.9 (240.6 – 481.6)
sG	Strongly informative priors	Vaccination	5.90 (4.44 – 7.42)	413.1 (290.1 – 541.3)

Scenarios	Changed variable	Interventions	BCR (95% CI)	NPV (95% CI), thousand CNY (Chinese Yuan)
	for vaccine efficacy	Combination	4.26 (3.24 – 5.32)	363.8 (250.0 – 482.0)
sH	Partial compensation	Test-and-culling	2.23 (1.67 – 2.79)	216.4 (118.6 – 316.2)
sI	Partial compensation	Vaccination	5.56 (4.06 – 7.15)	384.9 (258.5 – 518.6)
sJ	Partial compensation	Combination	3.98 (2.93 – 5.06)	332.3 (215.9 – 453.0)
sK	No compensation	Test-and-culling	2.02 (1.47 – 2.59)	180.0 (83.2 – 280.2)
sL	No compensation	Vaccination	5.29 (3.81 – 6.86)	362.2 (236.7 – 494.6)
sM	No compensation	Combination	3.72 (2.70 – 4.80)	304.0 (189.4 – 424.4)

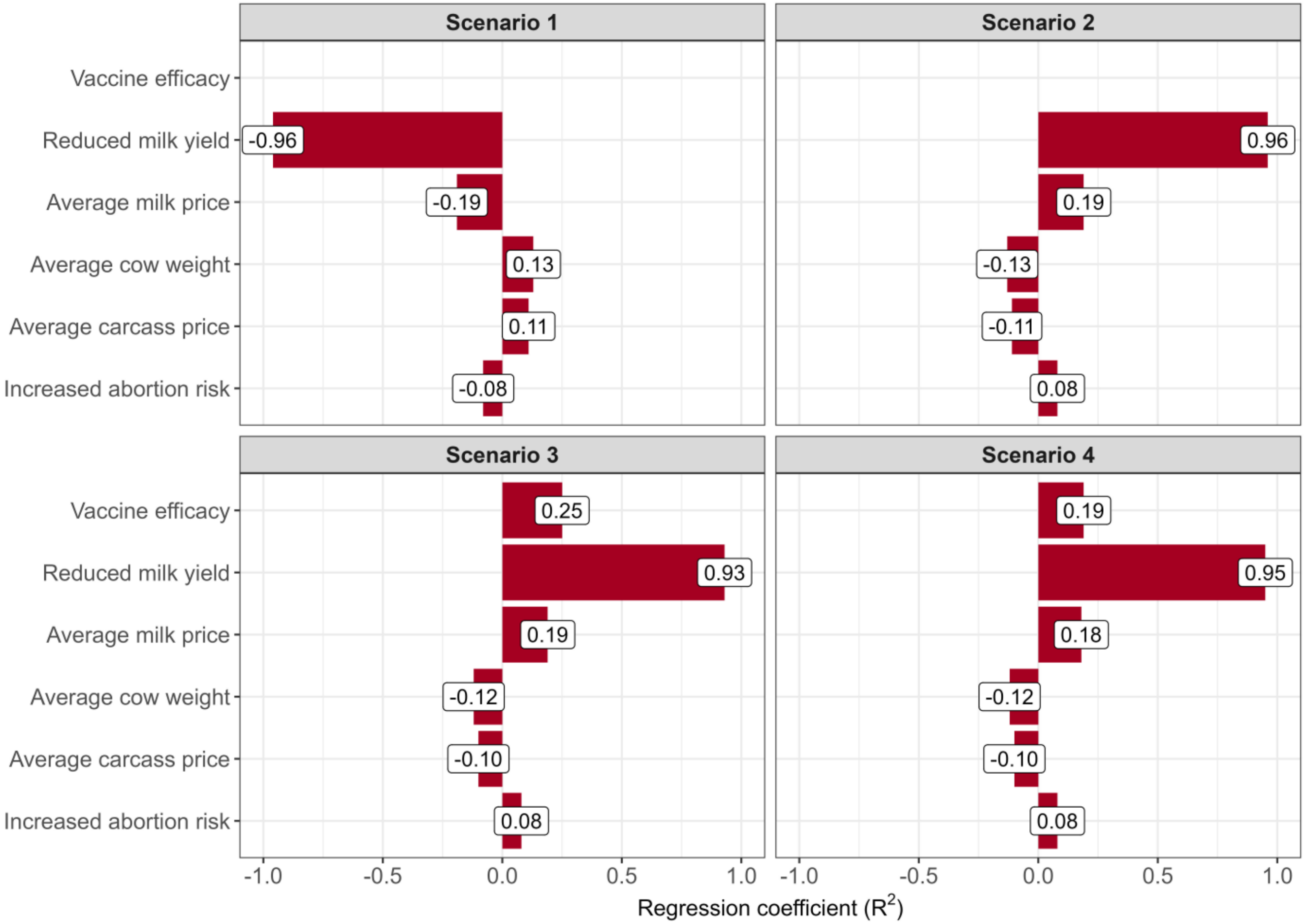

Figure 6. 5 Regression coefficients (R^2) of the sensitivity analysis for no intervention (Scenario 1), test-and-culling (Scenario 2), vaccination (Scenario 3), and combined control programs (Scenario 4).

6.5 Discussion

While several studies have reported the financial impacts of brucellosis on livestock globally (Kiiza et al., 2023; McDermott et al., 2013; Singh et al., 2015; Singh et al., 2018), to the best knowledge of authors, this is the first financial study to assess the economics of *Brucella* spp. and its interventions in the Chinese dairy sector. Our main findings suggest that *Brucella* spp. could lead to severe financial consequences on infected dairy herds, costing each infected cow 4,019 CNY or each animal 78.9 CNY annually. All three intervention approaches to *Brucella* spp. cost-effectively reduced disease prevalence and yielded more financial benefits than the related costs over ten years, as shown by positive NPVs and > 1 BCRs (Table 6.3). From an economic perspective, the optimal intervention approach among the three was Scenario 3 based on vaccination alone, as it produced the maximum BCR estimate. By quantifying the financial consequences and evaluating benefit-cost outcomes of different intervention strategies, we have addressed a critical knowledge gap in the field, providing valuable insights for farm stakeholders in the Chinese dairy sector.

Our findings, conducted with farmers as the primary stakeholders, estimated that each infected cow would suffer from a median economic loss of 4,019 CNY (95% CI: 2,910 – 5,147, equivalent to US$ 603, 95% CI: 437 – 772) per year, with milk loss accounting for approximately 70% of the total losses, highlighting the considerable risk of brucellosis for farm profitability and sustainability. The predicted economic losses for an infected cow in our study are much higher than US$ 50.8 per infected yak per year in Tibet yaks (Zeng et al., 2019b), which is highly likely due to differences in productivity, disease effects, and feeding systems between dairy cows and yaks. Intensively fed dairy herds likely generate greater production value primarily through milk yield and calving than yaks, amplifying their relative losses per individual infected animal. Santos et al. (2013) estimated that brucellosis caused an average of R$ 420.1 (equal to US$ 211.1 at that time) per infected cow per year in Brazil, lower than our estimate. This significant disparity is because our baseline milk production and milk price are two and three times those at their study time, respectively (Leite et al., 2018; Santos et al., 2013). This emphasizes that economic estimates are time-sensitive and context-specific and cannot be compared between absolute values without careful consideration (McDermott et al., 2013). A possible solution could explore the proportion of the financial impact of brucellosis in revenue at the farm level, which could be used to compare differences in financial impacts of the same disease between studies. However, the prerequisite is the need for reliable farm revenue data.

In this study, milk loss (70%) was the most predominant contributor to the overall losses due to *Brucella*, followed by increased replacement (8.8%) and pregnancy loss (8.6%) (Figure 6.3). This finding is consistent with previous reports in cattle (Santos et al., 2013; Singh et al., 2015) and yaks (Zeng et al., 2019b). Reduced milk production and pregnancy failure are the primary economic consequences of *Brucella* spp. infection in dairy cattle (Kiiza et al., 2023), adversely affecting farm sustainability and profitability. When scrutinizing milk losses, infection with *Brucella* spp. causes milk losses of 2,810 CNY per infected cow annually, approximately 9.6% of the expected average milk production value. Notably, we only considered the value of the calf for the abortion-related losses in the calculation, while other related side effects (e.g., reduced milk yield and replacement) were not included in the abortion losses to avoid double counting, thus attenuating the direct losses of abortion in our estimation. Our study has demonstrated that these components (Figure 6.1) of disease impact productivity and influence the accuracy of disease economic estimates (McDermott et al., 2013). Our longitudinal study (Chapter 5) provided population-specific and empirical information about the effects of disease on milk production and pregnancy in Henan dairy herds, refining the accuracy of our estimates. However, it is worth highlighting that our financial loss estimation (Scenario 1) was likely undervalued since losses indirectly related to the disease were not included in our analysis, such as the cost of isolation and treatment for aborted cows, increased conception failure, and repeated breeding, and increased labor burden (Montiel et al., 2015). These expenditures were unavailable, and it is challenging to distinguish brucellosis-related effects from other potential causes (e.g., bovine viral diarrhea virus) (Deng et al., 2020). Government expenditure in disease control and prevention, such as compensation for culled animals and vaccine procurement, is also a component of indirect disease losses, but it was not included in this assessment from a dairy herd perspective. Incorporating these losses in future studies would provide a more accurate economic assessment of the disease.

Only a cost-effective control program can sustainably enhance farmers' compliance with the proposed intervention approach. Our analysis revealed that the vaccination strategy (Scenario 3) yielded a best BCR of 5.84 (95% CI: 4.34 – 7.42), which is consistent with what was reported in other studies (Kiiza et al., 2023; Roth et al., 2003; Singh et al., 2018; Zeng et al., 2019b). Our estimate may be overestimated because this analysis did not incorporate other risks (e.g., disease introduction via introduction of new animals) and costs (e.g., positive animal slaughtering). Recent retrospective economic studies reported that a ten-year vaccination program achieved favorable benefits with BCRs of 2.96 (Ferreira et al., 2023) and 3.43 (Barros et al., 2023) in two states of Brazil, only considering private costs. Roth et al. (2003) established a sophisticated computational simulation model to estimate the human health benefits of mass livestock vaccination against *Brucella* spp. in Mongolia with a BCR of 3.2 (95% CI: 2.27 – 4.37). Human health benefits were not included in our analysis because they were beyond the scope of our study, and no accurate data indicated the proportion of human cases attributed to *Brucella* spp. originated from dairy cattle in China (Wang et al., 2024b). Control of *Brucella* spp. in dairy herds could improve the quality and safety of milk products and reduce the risk of humans acquiring *Brucella* spp. from dairy cattle and products. Therefore, we expect that human health would also benefit from interventions for *Brucella* spp. in dairy herds in China.

Our results for test-and-culling intervention (Scenario 2) suggested that it was also economically favorable (BCR = 2.43) but less cost-effective than vaccination intervention alone. In contrast, Zeng et al. (2019b) found that the test-and-culling strategy in Tibetan yaks was not economically favorable (BCR = 0.21), which is likely due to the more significant expenditure of eliminating positive animals and purchasing replacement heifers relative to vaccination alone. Holt et al. (2023) utilized a simulation model to demonstrate that test-and-culling could suffer from imperfect diagnostic tests, resulting in unnecessary expenditure in culling false-positive cows. Furthermore, test-and-culling intervention is also challenging to implement and gain support from farmers' perspectives in low-income regions or countries (Dhand et al., 2021) since compensation for slaughtered animals is often below market value (Department of

Agriculture and Rural Affairs of Henan Province, 2022b) and underlying benefits from the removal of apparently healthy cows are rarely visible for farmers. The availability of *Brucella*-free replacement heifers and farmers' acceptability of control strategies are worthy of consideration before implementing such control programs (Dhand et al., 2021). In our questionnaire survey (Chapter 4), we also found that Henan dairy farmers preferred to adopt vaccination rather than test-and-culling due to the substantial operating costs.

The combination of test-and-culling and vaccination is controversial in *Brucella* spp. control programs due to the lack of a vaccine that can differentiate infected from vaccinated animals (DIVA). Our Scenario 4 is to conduct test-and-culling in the initial year, followed by vaccination of replacement heifers, producing a BCR of 4.23 (95% CI: 3.19 – 5.31) over ten years. Zeng et al. (2019b) estimated the BCR of the combination of vaccination and test-and-culling at 0.20 (95% CI: 0.13, 0.30); however, they did not clarify how vaccination would be used in conjunction with test-and-culling and the problem of serodiagnostic interference posed by vaccination. Yang et al. (2021) recently developed a DIVA vaccine and related differential diagnostic test that has been licensed in dairy cows in China; however, the results of field trials on the protection efficacy of this DIVA strain (*B. abortus* A19ΔvirB12) against virulent *Brucella* spp. and abortion and its differential diagnostic performance still deserve further extensive investigation through a double-blind randomized trial. Therefore, we did not consider using the *B. abortus* A19ΔvirB12 strain in this study. Other joint use of vaccination and test-and-culling applied in successfully eradicated countries are to first employ vaccines to reduce the true prevalence of *Brucella* spp. in the herd, followed by termination of vaccination and implementation of strict test-and-culling (Zhang et al., 2018). Future studies could explore this scenario in China.

The sensitivity analysis results confirmed that all interventions were still cost-effective and had a BCR of at least 2.02 for all scenarios, even without government compensation for farmers (Table 6.4). This implies that our results are robust; altering some assumptions or parameters in the model did not affect the overall conclusion that vaccination remains the most financially beneficial strategy to control brucellosis over the ten years for a typical dairy herd in Henan. However, there are no published randomized double-blind trials on the protection of the *B. abortus* A19 strain against infection or abortion, so we compromise using parameters derived from the highly homologous *B. abortus* S19 strain (de Oliveira et al., 2022; Wang et al., 2022). Although the sensitivity analysis demonstrated the robustness of the results, accurate parameters from field trials about the protection of the A19 strain in dairy cows could refine our financial estimation. For the test-and-culling strategy, a parallel testing scheme, characterized by improved overall sensitivity and reduced specificity, reduced the BCR to 1.55. The BCR reduction was most likely associated with increased culling costs related to more false positives. Regression analysis indicated that BCR was most sensitive to the reduced milk yield of infected for all scenarios, followed by the vaccine efficacy for the vaccination control strategy. Our longitudinal study (Chapter 5) provided population-specific disease effects on milk yield and pregnancy outcomes, which could improve the accuracy of our analysis. However, for other populations where feeding practices and production levels are incompatible with our studied areas, we recommend estimating population-specific disease impact parameters first, remarkably reduced milk yield, to enhance the robustness of financial assessment.

While our main findings are robust, we acknowledge that there are some areas worth improving in future studies. The dynamic nature of disease transmission and intervention effects, coupled with demographic changes within dairy populations, may influence the accuracy of financial estimates. Some adult cows may be culled prematurely, resulting in a younger age structure and affecting overall herd productivity. Secondly, the risk of shedding *Brucella* spp. in infected cows is unlikely to remain constant throughout life (England et al., 2004); therefore, incorporating variable shedding risk would benefit the accuracy of the estimate. The introduction of new animals is an essential risk factor for the spread of *Brucella* spp. among Chinese dairy herds (Wang et al., 2024b); however, our assumption that all purchased animals are *Brucella*-free is challenging in China. Farmers' attitudes and behaviors toward disease control shape the adoption and maintenance of measures, so incorporating this heterogeneity into the assessment is desirable (Hill et al., 2023). Future studies could explore incorporating demographic dynamics, the risk related to animal procurement, stage- and age-specific disease impacts, and farmer's behaviors to enhance model accuracy (Hill et al., 2023; Holt et al., 2023).

It is worth highlighting that this study estimated the benefits and costs attributable only to this disease in dairy cows based on a Henan dairy farming setting from a farmer's perspective. The brucellosis control costs associated with slaughtering positive animals, animals, disease surveillance, and government compensation, as well as the public health benefits, are also worth investigating from a government perspective, especially when faced with multiple resource-competing zoonoses in China, such as Influenza A (H7N9) virus (Tang et al., 2022b) and rabies virus (Miao et al., 2021). Nonetheless, it can be confirmed from this study and others (Roth et al., 2003; Singh et al., 2018; Zeng et al., 2019b) that the benefits of vaccine use outweigh the costs, encouraging dairy farmers to adopt a vaccination approach to control *Brucella* spp. in China.

6.6 Conclusions

Given that the financial impacts of *Brucella* spp. in a typical infected dairy herd in Henan Province have been estimated at 4,019 CNY (95% CI: 2,910 – 5,147) per infected cow per year, the overall financial impacts of *Brucella* spp. at the provincial or national level would be likely significant based on the current high prevalence of *Brucella* spp. and sizeable dairy cattle populations in China. Our financial analyses suggest that *Brucella* spp. control programs through the vaccination of the entire population with annual boosters on replacement heifers on dairy herds is the most financially beneficial intervention approach at the farm level with a BCR of 5.84 (95% CI: 4.34 – 7.42). Considering the economic significance of *Brucella* spp. in dairy herds and the current favorable policy support for vaccination, we believe our recommendation would enhance farmers' adoption of intervention measures against *Brucella* spp.

6.7 Acknowledgments

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Chapter 7 General Discussion

7.1 Introduction

7.1.1 Aims of this chapter

This chapter integrates the key findings of this thesis, incorporating perspectives from disease diagnosis, farm biosecurity, epidemiology and disease impacts, and disease economics. The discussion contextualizes these findings within the broader framework of *Brucella* spp. control programs, assessing their implications for different stakeholders. Finally, this chapter will also highlight key knowledge gaps and propose future research directions to strengthen the effectiveness of brucellosis prevention and management in China.

7.1.2 Aims of this thesis

The main objective of this thesis is to provide evidence-based epidemiological information on *Brucella* spp. in intensively housed dairy herds in Henan Province, China. In Chapter 1, five research objectives were presented with the overall aim of tackling critical knowledge gaps affecting the effectiveness of the brucellosis control programs in Henan Province, China. These objectives were addressed through a series of studies. A One Health approach, efforts from different stakeholders, limitations, and future research directions are discussed.

7.2 *Brucella* spp. control implications

7.2.1 Overview of key findings

The first research objective was addressed in Chapter 2, where we identified *B. abortus* biovar

3 as the primary *Brucella* spp. for bovine brucellosis in Chinese dairy herds, whereas *B. melitensis* biovar 3 was more frequently isolated from humans in China from retrieved publications between 2004 and 2022 (Wang et al., 2024b). The median of animal-level apparent prevalence was 2.1% (range: 0.0% – 13.5%), while the median herd-level apparent prevalence was 10.2% (range: 0.0%–100%). Heterogeneities were observed across retrieved studies in terms of study design, geographical distribution, and transparent data reporting. Specifically, these reported prevalence estimates have not been adjusted for diagnostic sensitivity and specificity and are likely to mislead stakeholders about the true prevalence. Half of the retrieved studies omitted to report herd-level prevalence, which may cause stakeholders to underestimate the overall extent of brucellosis at the herd level. Addressing *Brucella* spp. in dairy cattle requires a coordinated and systematic approach to inform herd-level and regional control strategies that are both feasible and economical.

The limited application of diagnostic test performance in epidemiological analyses of brucellosis in China was highlighted in Chapter 2. Chapter 3 addressed this gap by evaluating the diagnostic performance of four serological tests and estimating the true prevalence of brucellosis. A novel Bayesian latent class model was developed to evaluate two binary and two continuous tests in a single analytical framework without a “Gold standard” test, incorporating additional fixed (parity) and random (farm) effects (Wang et al., 2024a). The optimal cut-off values of two continuous tests and the diagnostic sensitivity and specificity of four tests were determined by maximizing the Youden Index. The median true prevalences in the two sampled regions were estimated at 4.7% and 30.3%, respectively. Parity-specific odds ratios for positive serological status ranged from 1.2 to 2.2 for different parity groups compared to primiparous cows. These findings can directly support brucellosis control programs in Henan Province by improving disease diagnostic strategies, enabling more accurate case identification for test-and-culling programs, and refining surveillance efforts. Adjusted prevalence estimates offer a more accurate picture of disease burden, guiding targeted interventions in high-risk farms and areas. Additionally, applying validated diagnostic performance metrics to the subsequent longitudinal study and financial analysis improves the accuracy of disease impact assessments and the financial benefits and costs of control strategies.

To investigate the farm biosecurity practices and motivations of farm stakeholders (Research Objective 3), Chapter 4 analyzed data from a closed questionnaire survey conducted among dairy farm stakeholders in Henan Province. Overall, the adoption of biosecurity practices was inadequate and varied across dairy herds. Using multiple correspondence analysis and agglomerative hierarchical cluster analysis, two distinct farm clusters were identified. Dairy herds in Cluster 1, which demonstrated better adherence to the recommended biosecurity practices than herds in Cluster 2, were associated with medium to large herd sizes and higher educational levels among farmers. In contrast, Cluster 2 exhibited lower biosecurity compliance, with financial constraints emerging as both a primary barrier and motivator for biosecurity adoption. These findings highlight the need for targeted training to improve biosecurity practices, particularly in small dairy herds with limited resources. Demonstrating that farm biosecurity or other interventions improve farm productivity and financial benefits could effectively help farm stakeholders comply with recommended measures.

The incidence of seroconversion to *Brucella* spp. and its effect on dairy cattle productivity help understand the disease epidemiology and impact but had not been previously studied in China. Chapter 5 addressed this knowledge gap through a one-year longitudinal study, estimating an annual incidence of seroconversion of 13.1% in Henan dairy herds. Cows that seroconverted or remained test-positive produced 2.8 to 3.2 kg/day less milk than consistently negative cows. Seroconverted cows (RR = 4.26, 95% CI: 3.17 - 5.73) and consistently positive cows (RR = 3.25, 95% CI: 2.39 - 4.42) had a significantly increased risk of abortion compared to consistently negative cows. Elevated somatic cell count was positively associated with seroconversion and consistently seropositive to *Brucella* spp. These findings quantified the significant impact of *Brucella* spp. on Henan dairy herds and could directly motivate farm stakeholders to adopt countermeasures.

Based on these research findings and data from previous chapters, the knowledge gap (Research Objective 5) around the financial costs and benefits of brucellosis was addressed in Chapter 6. A financial analysis was conducted for *Brucella* spp. and three intervention measures in a typical Henan dairy herd. The direct financial losses of *Brucella* spp. were estimated at 78.9 (95% CI: 57.2 – 101.1) Chinese Yuan (CNY) per animal and 4,019 CNY (95% CI: 2,910 – 5,147) per infected animal annually. Furthermore, vaccination was identified as the most cost-effective intervention for a typical dairy herd, offering the highest benefit-cost ratio (BCR) of 5.84 (95% CI: 4.34 – 7.42) and net present value (NPV) of 408.2 thousand CNY (95% CI: 281.9 – 541.2 thousand CNY) after ten years. Sensitivity analysis demonstrated the robustness of the conclusions despite the changes in some parameters and assumptions, including government financial contributions. These findings provide strong financial justification for investing in brucellosis control programs in Henan. By quantifying the direct costs of *Brucella* spp., policymakers and farm stakeholders can better understand the financial burden of the disease and the potential financial benefits of interventions.

7.2.2 One Health Approach

Brucella spp. represents a classic One Health challenge, impacting the dairy industry, farmers, food safety, and public health in China and globally (Moriyón et al., 2023). Effective control requires a multisectional collaboration among farmers, veterinarians, researchers, governmental veterinary departments, and public health departments. Current brucellosis control programs in China are primarily government-led, with limited active engagement from farm stakeholders, such as limited adoption of farm biosecurity practices (Chapter 4). While farmers in Henan Province generally supported brucellosis control policies, they faced significant barriers to implementation, including financial constraints, inadequate scientific guidance, and a shortage of professional veterinarians. Chapter 6 evaluated hypothetical ten-year farm-led brucellosis vaccination and test-and-culling programs supplemented by government financial compensation. The results demonstrated that vaccination was the most financially beneficial intervention, offering substantial economic benefits for farm stakeholders and potentially reducing public health risks. This underscores the need for an integrated control strategy that aligns financial benefits with disease control efforts.

A new definition of One Health emphasizes an integrated approach involving domestic and wild animals, humans, and the environment (including ecosystems) at multiple levels (One

Health High-Level Expert et al., 2022). The steps and challenges of implementing a One Health approach are elaborated in Pepin et al. (2024). Our findings and others (Moriyón et al., 2023) confirm that *Brucella* spp. is a One Health problem to both humans (Chapter 2) and dairy cows (Chapters 5 & 6) in China, and other studies have also demonstrated its threat to the environment, pets, and wildlife (Xue et al., 2023; Yan et al., 2022; Yang et al., 2024). *Brucella* spp. threatens the environment, pets, and wildlife through persistent or intermittent shedding in animal secretions, contaminating soil, water, and pasture. Infected wildlife and stray animals can act as reservoirs, sustaining the pathogen in ecosystems and enabling spillover back to domestic livestock and humans, compromising eradication efforts and increasing public health risks (Kamath et al., 2016; Wareth et al., 2017). Given these risks, a One Health approach should integrate existing control programs across sectors to enhance collaboration and optimize disease management strategies (One Health High-Level Expert et al., 2022). Brucellosis programs in Malta and Serbia have demonstrated the added values provided by the One Health approach in achieving brucellosis eradication after multiple failed attempts at eradication due to fragmented and uncoordinated efforts (Buttigieg et al., 2018). The successful implementation of the One Health approach depends on the enforcement of both existing and new legislation, requiring close collaboration among all stakeholders related to brucellosis, including public health, veterinary authorities, policymakers, farmers, and researchers. Therefore, what dairy farmers, researchers, and governments can do to build an integrated One Health approach against brucellosis will be discussed.

7.2.3 The role of dairy farmers

Farmers are critical of *Brucella* spp. and other infectious disease control programs, as their management practices can directly influence disease transmission, farm biosecurity, herd health, and production. However, our findings indicated the heterogeneous adoption of farm biosecurity measures among investigated dairy herds in Henan Province (Chapter 4). Maintaining a closed farm is the most effective prevention and control approach for brucellosis and other pathogens (Robertson, 2020), but this approach may not always be feasible for most dairy herds. Our survey (Chapter 4) found that 60% of selected dairy herds introduced new animals without quarantine and prior testing in 2021-2022. Feeding raw milk to calves is a well-known risk factor for the persistence of *Brucella* within the dairy population (Capparelli et al., 2009; Khurana et al., 2021; Wainaina et al., 2020) since infected cows can excrete viable *B. abortus* into milk lifelong (Capparelli et al., 2009). However, only 15% of investigated herds used boiled or pasteurized raw milk to feed calves (Chapter 4). These results may suggest a lack of perceived awareness of the transmission pathways of *Brucella* spp. in dairy herds or other reasons such as a lack of facilities and labor sources. Educating farm stakeholders on the transmission pathways of *Brucella* spp. is crucial to increasing their understanding of the risks of various practices. However, education alone may not be sufficient to drive behavioral change in farm management. Providing practical management guidelines and financial information, such as biosecurity protocols, proper animal handling techniques, and financial burden, can help farmers adopt effective disease prevention measures.

The longitudinal study (Chapter 5) estimated the production impacts of *Brucella* spp., with seroconverted cows producing 2.8-3.2 kg/day less milk and exhibiting higher abortion risks (RR = 4.26, 95% CI: 3.17, 5.73) compared to consistently negative cows. These findings were further used to evaluate the financial costs and benefits of control programs in Chapter 6, highlighting the need for continuous disease monitoring and data recording. However, poor on-farm record-keeping remains a major limitation in disease management in our longitudinal study and others (Chen et al., 2021). In China, many farmers undervalued disease data collection and were concerned about reputation damage (Chen et al., 2021), despite its potential to improve disease management and optimize herd production performance. Our research, collaborating with the Henan Dairy Herd Improvement (DHI) Centre, demonstrated that high-quality data significantly enhanced epidemiological assessments and decision-making processes. Such research could enhance farmers' understanding of the impact of disease on production and underline the benefits of data recording. Furthermore, we have demonstrated that the financial benefits of adopting intervention measures are expected to exceed the costs of measures even without government compensation against brucellosis for dairy herds in Chapter 6. This valuable information could increase the confidence of farm decision-makers in investing in *Brucella* spp. control programs.

7.2.4 The role of researchers

Researchers play a crucial role in disease control programs in China given their findings can directly or indirectly inform stakeholder decisions on disease management. While they may not directly be involved in implementing control programs, their contributions are essential in developing evidence-based policies, improving diagnostic tools, evaluating vaccine efficacy, modeling disease spread, and assessing the effectiveness of intervention strategies. However, current studies in veterinary epidemiology in China suffer from heterogeneous study designs and reporting quality. Chapter 2 revealed that existing problems in published literature included a lack of sampling details, power analysis, appropriate statistical methods, and detailed and transparent reporting of herd-level prevalence and geographic information (Wang et al., 2024b). Sargeant et al. (2016) introduced the STROBE-Vet (Strengthening the Reporting of Observational Studies in Epidemiology – Veterinary), the internationally recognized reporting guidelines for veterinary epidemiological observational studies. O'Connor et al. (2016) provided additional explanation and elaboration for the STROBE-Vet, which can serve as a benchmark for prospective veterinary epidemiological research reporting. Other standard guidelines for specific research areas, such as STARD-BLCM for Bayesian latent class models (Kostoulas et al., 2017) and PRISMA-P for systematic review and meta-analysis (Moher et al., 2015), are also accessible. Intrinsic biases in research due to sampling strategy and study design should be highlighted rather than hidden. Promoting and adhering to these scientific guidelines among Chinese veterinary researchers should be a priority for academics.

Bias is an inherent challenge in epidemiological studies, influencing the validity and interpretation of study findings (Dohoo et al., 2009). In China, *Brucella* spp. research faces several methodological limitations that may impact the accuracy and generalizability of findings. Selection bias is a significant concern, as many Chinese studies are limited to dairy herds within specific databases and provinces, such as the Henan DHI program, while herds outside these systems remain understudied. For example, Chapter 2 identified spatial heterogeneity in studies reporting *Brucella* spp. from both dairy cattle and humans. Expanding research efforts in unsampled and underreported areas and dairy herds would provide a more comprehensive understanding of prevalent *Brucella* spp., true prevalence, and risk factors, despite logistical challenges, such as access restrictions and data-sharing barriers. Measurement and record bias is another critical problem, as it can significantly influence the research findings. The key sources for measurement bias include diagnostic errors due to imperfect diagnostic performance, non-random selection of dairy herds and animals, and observer bias. These biases have been discussed in previous chapters and should be strengthened in future studies. Studies relying on self-reported farm records or prioritizing herds with more complete data could introduce systematic biases, skewing results toward better-managed farms. Also, access to quality production data is challenging in China due to concerns about data security and privacy protection. Recall bias and social desirability bias tend to occur in questionnaire and interview surveys since farm stakeholders may subconsciously tend to answer positively due to concerns about culling policies, economic losses, or social stigma (Gilbert et al., 2014). Government brucellosis surveillance data are underreported or delayed, especially in remote or resource-limited regions in China (Wang et al., 2021b). To validate findings, triangulation through researcher observations should be incorporated, allowing for objective validation of self-reporting practices. Future studies could further enhance accuracy by integrating direct assessments, follow-ups, or electronic monitoring of biosecurity measures. Addressing these shortcomings would improve the reliability of *Brucella* research and inform more effective disease control strategies in China.

Veterinary epidemiological studies have evolved from traditional observational studies to multidisciplinary research, including pathogen biology, biosecurity, statistics and computer science, mathematical modeling, economics, social behavior, psychology, and network topology (Hill et al., 2023). However, most Chinese veterinary studies still focus on the development of diagnostic tests, vaccines, and pathogenic mechanisms, with less emphasis on epidemiological evidence and non-pharmaceutical tools. While these studies on biological mechanisms advance science and novel tools, they often take a long time and cannot solve emergency problems immediately. For instance, African Swine Fever (ASF) invaded the Chinese pig industry, causing significant and ongoing losses (Cheng et al., 2022). Except for a self-authorization vaccine in Vietnam, the ASF vaccine has not yet been authorized globally despite the ASF was found more than one century ago. Manipulable risks and proactive factors should be of particular interest to stakeholders but receive less attention in the disease decision-making process in China.

Researchers also bridge the gap between animal health, human health, and environmental factors, ensuring that a comprehensive understanding of disease, dairy farming, and socioeconomics informs control measures. Currently, there are gaps between brucellosis control policy formulation and true compliance. Our survey found that most respondents supported the current brucellosis control policy but suggested that it was difficult to comply fully with recommendations, such as a ban on live cattle movement or trade between vaccinated and non-vaccinated areas in China (Chapter 4). This regulation is favorable from an epidemiological perspective but commercially and operationally challenging for farmers. A sound policy must consider the interests of all parties involved in programs before it can take effect (Zinsstag et al., 2011). In addition, there is a lack of collaboration between veterinary and public health researchers in China. Information barriers, especially regarding the causative *Brucella* spp. and contact history with specific livestock, hinder establishing effective epidemiological links between human and livestock cases and eliminating infection sources. Effective collaboration between veterinary and public health researchers is essential to achieve the shared goal of controlling brucellosis in livestock and humans. Practical steps to enhance this collaboration include (1) establishing integrated reporting and surveillance systems that link human and animal brucellosis cases and utilize GIS-based mapping tools to visualize hotspots and overlapping areas; (2) strengthening joint field investigations and data sharing; (3) demonstrating the added values of stronger collaboration than fragmented efforts.

7.2.5 The role of government

Government-led *Brucella* spp. control programs provide unified coordination, standardized protocols, and regulatory enforcement, aiming for broad participation across dairy herds, regardless of size or location. However, effective implementation requires collaboration among multiple stakeholders, including dairy farmers, researchers, and veterinary and public health agencies. The government plays a central role in interdepartmental coordination, resource allocation, and policy development, while the specific implementation requires engagement from industry, farm stakeholders, and research sectors. Demonstrating the long-term and sustainable benefits of brucellosis control programs for all parties at different levels will help to engage all stakeholders to enhance the overall effectiveness of control programs.

Surveillance and reporting systems are essential government responsibilities, requiring efficient and integrated mechanisms for capturing accurate, real-time data on animal and human brucellosis cases. Wang et al. (2021b) found different trends in the number of brucellosis notifications between human and animal systems in China, suggesting that there is a high likelihood of underreporting in the animal notification system. However, the National Health Commission and the Ministry of Agriculture and Rural Affairs separately manage the surveillance and notification systems for humans and animals, respectively, in China. Streamlining and integrating these notification systems, alongside leveraging geospatial mapping and data analytics, would improve surveillance efficiency and response coordination. Transparent and timely reporting mechanisms could inform all stakeholders, from dairy farmers to the public, ensuring a coordinated response to emerging outbreaks. A five-year action plan for brucellosis (2022-2026) has been set at national and provincial levels to achieve the desired outcomes (Ministry of Agriculture and Rural Affairs, 2022), with Henan Province aiming to control the prevalence of brucellosis in livestock to 0.4% and 7% at the individual and farm levels by 2026 (Department of Agriculture and Rural Affairs of Henan Province, 2022a). Governments and researchers should design an achievable and feasible target and provide necessary guidance and resources to help farmers complete expected goals at both animal and herd levels.

Financial and technical support is another key governmental responsibility, necessitating financial assessments of brucellosis control programs to optimize resource allocation. Roth et al. (2003) demonstrated human health benefits from vaccination campaigns against brucellosis in livestock, offering a cost-benefit ratio of 3.20 (95% CI: 2.27 – 4.37) and a net present value of US$ 18.3 million. Our survey (Chapter 4) highlighted that financial incentives remain the strongest motivation for dairy farmers, as production gains from vaccination or other control measures are not directly visible to farmers. Therefore, sustained government subsidies for vaccination, diagnostic testing, and farm biosecurity improvements are crucial to alleviating farmers' financial burden and promoting compliance. The effectiveness of such financial support requires continuous monitoring and evaluation to ensure that resources are being used efficiently and reaching those in need of brucellosis control programs.

Raising public awareness about brucellosis is crucial for adopting proactive measures to prevent the disease. Government-led campaigns should be culturally tailored and evidence-based, addressing the unique needs and perceptions of different communities (Dhand et al., 2021). Essential messaging should emphasize *Brucella* spp. transmission pathways, clinical manifestations, and proper management practices. Thoughtful campaign design can incorporate available scientific evidence and evaluate the benefits of farmers' behaviors in infectious disease control (Hill et al., 2023). Providing farmers with information about the production benefits of changing behaviors can directly contribute to increased compliance with recommended measures. Farmers need to be informed that vaccination alone does not provide complete protection against *Brucella* spp. and should be complemented by other measures such as adequate biosecurity measures.

7.3 Future research needs

Despite the valuable information provided in this thesis, there are still many knowledge gaps and questions pending to be answered for the control and elimination of *Brucella* spp. in China. Herd-level prevalence estimations are still limited in China (Chapter 2), hindering a comprehensive understanding of the epidemiology of *Brucella* spp. at the herd level. Additionally, most diagnostic evaluation and validation studies have focused on the diagnostic sensitivity and specificity at the animal level and overlooked performance at the herd level. In disease surveillance, determining a minimum sample size within the individual herd is often encountered when dealing with dairy herds of different herd sizes. The minimum sample size within each dairy herd can be determined to ensure sufficient sensitivity and specificity of disease surveillance (Stevenson, 2021). Characterization of cattle movement patterns, which could inform potential spread routes of *Brucella* spp. and more effective disease surveillance at the herd level, remains unexplored in China. As an allocator of public resources, the government needs to justify that investments in *Brucella* spp. control programs are financially beneficial. Beyond eliminating susceptible groups through vaccination, identifying infection sources and spillover routes from livestock to humans has always been crucial for breaking transmission pathways from a traditional epidemiological perspective. Modern molecular diagnostic and bioinformatics technologies can give us more detailed epidemiological information for disease control programs. These knowledge gaps will be discussed in detail to provide implications for enhancing *Brucella* spp. control programs in China.

7.3.1 Herd-level diagnostic evaluation and prevalence

Diagnostic test evaluation is a cornerstone of implementing any disease control and elimination programs globally (WOAH, 2023a). As most control measures are conducted on a farm basis, identifying positive farms is a prerequisite for understanding the epidemiology of the disease and implementing control measures at the herd level. Bulk milk samples or several individual samples collected within a herd are used to identify positive farms or demonstrate disease freedom given the designed herd-level sensitivity and specificity (Nobrega et al., 2023). While Chapter 3 provided a detailed evaluation of four serological tests at the individual animal level, their performance at the herd level remains unexplored in China. Furthermore, optimizing the minimum number of samples required to achieve desired levels of herd-level sensitivity and specificity is an area that warrants extensive research. Arif et al. (2018) previously evaluated three serological tests for diagnosing bovine brucellosis for Pakistani smallholder farms at the farm level, and Lurier et al. (2021) developed a novel methodology to optimize sample size by maximizing the sum of diagnostic sensitivity and specificity at the herd level. Herd-level sensitivity and specificity are also used to demonstrate the probability of disease freedom in control programs at a regional or national level (Toftaker et al., 2020). Pei et al. (2025) used individual test data to demonstrate the remarkable progress of *Brucella* spp. elimination programs in goat flocks of Hubei Province but also emphasized that the lack of flock-level data affected the uncertainty of results. In addition, a better understanding of test performance is essential for defining robust farm-level case definitions, as relying on a single positive test result may lead to misclassification given imperfect specificity (Greiner et al., 2000a). Farm classification should incorporate herd-level prevalence thresholds, and longitudinal surveillance, ensuring more robust positive/negative farm classification and accreditation of brucellosis-free status (Christensen et al., 2000). Providing such information improves the efficiency of routine disease surveillance and identification of positive farms and potentially reduces the amount of sampling required for a given diagnostic performance.

Herd-level prevalence is an essential epidemiological metric for understanding disease in a regional or national population. Herd-level prevalence is usually defined as the proportion of farms containing at least one positive animal in a sampled population; hence, a tangible decline is more difficult to achieve than the animal-level prevalence in a typical farm. Also, herd-level prevalence is influenced by the number of samples within one farm and individual-level diagnostic sensitivity and specificity. However, about half of the studies in retrieved literature did not report herd-level prevalence of *Brucella* spp. in dairy herds in China (Wang et al., 2024b), suggesting that herd-level prevalence is likely to be overlooked compared to animal-level prevalence in China. Herd-level prevalence data has essential implications in informing evidence-based control measures and providing valuable feedback on the effectiveness of disease control programs. For instance, Shaukat et al. (2024) used bulk tank milk samples to determine the herd-level prevalence of three infectious pathogens in Canada and identify risk factors associated with these pathogens at the herd level. Such information could be imperative in identifying hotspot areas for further investigation and guiding policy-making to improve animal health.

7.3.2 Cattle movement network

Cattle movement or trade is a critical risk factor driving the between-herd and interprovincial spread of many diseases in China, significantly influencing the spread of *Brucella* spp. and other infectious diseases. While risk-based disease surveillance informed by animal movement networks has been shown to enhance disease control capability in the Chinese poultry and pig industries (Li et al., 2020a; Martin et al., 2011; Tang et al., 2022a), similar studies focusing on cattle movement network are notably absent in China. Compared to the swine and poultry sectors, where diseases such as African Swine Fever (ASF) and H5N1 avian influenza have resulted in stringent management measures, the cattle sector has relatively less comprehensive and sufficient data collection within existing Chinese livestock tracking systems. In addition, cattle movements are less frequent than pigs and poultry in China, limiting data availability for comprehensive network analysis. Despite these challenges, the significant role of cattle movement in the spread of brucellosis has been identified in China (Wang et al., 2024b) and other countries (Rodrigues et al., 2023). Incorporating the cattle movement or trade network into the infectious disease model could inform brucellosis control strategies to prioritize implementing interventions on highly connective farms that may serve as hotspots for disease transmission network (Marquetoux et al., 2016; Savini et al., 2017). Future studies can provide more robust evidence to guide disease surveillance from investigating cow movement patterns and characteristics combined with epidemiological data.

7.3.3 Economic benefits for public health from brucellosis control in animals

Controlling brucellosis in animals reduces disease-related expenditures in livestock and significantly benefits human health by decreasing the spillover risk (Roth et al., 2003). Such public health benefits represent an indirect but critical benefit from government-led animal brucellosis control programs. The recent increase in human brucellosis notifications identified in Chapter 2 likely reflects a real rise in incidence, as the upward trend persisted despite the

COVID-19-related disruptions to control efforts after 2020. However, reporting biases, such as improved awareness among farm workers and healthcare providers, may have also contributed to higher detection and notification rates (Liu et al., 2025). The limited scope of the human brucellosis notification system hinders a precise assessment of the relative contributions of true incidence increase versus increased reporting, underscoring the need for enhanced surveillance and epidemiological investigations.

In Chapter 6, while we explored the financial viability of various control strategies from a farmer's perspective, identifying vaccination as the most financially beneficial option, a broader analysis from the government's standpoint remains unexplored in China. The human health burden of brucellosis was estimated at 5,741 disability-adjusted life years (DALYs, 0.00413 per 1,000 population), and the indirect economic burden was about 31.29 million US dollars in 2017 in China, based on officially notified human cases (Peng et al., 2020b). It is worth noting that this notification number is likely to be underestimated due to potential underreporting and misdiagnosis, as a recent study estimated the annual global incidence to be 2.1 million, far exceeding earlier projections (Laine et al., 2023; Pappas et al., 2006). In Armenia, Anyanwu et al. (2024) recently found that the upgraded test-and-slaughter of livestock only had a cost-benefit ratio (0.133) for controlling human brucellosis at 80% compensation and 5% discount rate over a decade and the cost-effectiveness ratio at US$ 1,587 per DALY averted (95% CI: US$ 1,268–US$ 2,009). Conversely, Roth et al. (2003) demonstrated in Mongolia that mass vaccination of livestock yielded a benefit-cost ratio of 3.2 (95% CI: 2.3, 4.4) and a cost-effectiveness of US$ 19.1 per DALY averted (95% CI: 5.3, 486.8) for human health over a decade. Given the substantial increase in the incidence of human brucellosis in China over the past twenty years (Wang et al., 2024b), further studies are warranted to assess the true incidence of human cases and economic burden of human brucellosis in China. By addressing these areas, we can provide a more holistic understanding of the financial benefits of controlling brucellosis in humans and support the development of more effective public health policies in China.

7.3.4 Molecular Epidemiological Study

The scope of this thesis has not extended to bacterial isolation, identification, or the molecular epidemiological analysis of *Brucella* spp. within dairy herds in Henan Province. This limitation restricts our understanding of the diversity and epidemiology of *Brucella* spp. currently circulating in this province. The major assumption that *B. abortus* is the predominant *Brucella* species circulating in Henan dairy herds warrants further investigation, especially monitoring of cattle processed at slaughterhouses and aborted cows in dairy herds. Despite the recognized prevalence of brucellosis among dairy cattle (Chapter 2), the isolation of *Brucella* spp. in dairy cattle is predominantly reported from only a few provinces, with significant gaps in many provinces (Wang et al., 2024b). Therefore, there is a critical need to increase efforts in isolating and genetically characterizing *Brucella* spp. from dairy herds across Henan and other underreported provinces. This would involve employing advanced molecular techniques to understand the diversity and distribution of *Brucella* spp. better. Following the methodology developed by Kamath et al. (2016), who used advanced molecular epidemiological tools to reveal the *B. abortus* transmission routes between wildlife and domestic animals in Greater Yellowstone, similar studies in China could identify the pathways of *Brucella* spp. transmission among local herds and possibly among small ruminants, wildlife, and dairy cattle. Furthermore, isolated *Brucella* spp. can be used to develop new vaccine candidates that are more effective against local *Brucella* spp. These *Brucella* spp. can also be utilized in experimental challenge trials to evaluate vaccine efficacy against *Brucella* spp., tailoring vaccination strategies to the specific *Brucella* spp. prevalent in the study area. Recent advancements from phylogenetics to phylodynamics highlight that integrating phylogenetics with infectious disease epidemiology can enhance our understanding of the transmission dynamics and direction and identify appropriate interventions (Guinat et al., 2021). Janke et al. (2023) used genomic analysis to trace *B. abortus* origins to Africa, from where it spread to Western Europe and Asia. In China, Zhu et al. (2020) examined a total of 1382 *B. melitensis* isolates collected between 1955 and 2018 from humans and animals across 29 provinces of China, revealing that southern *Brucella* spp. likely originated from northern China, driven by the movement of infected small ruminants and products. These findings underscore the interconnected nature of brucellosis transmission across regions and hosts, highlighting the need for an integrated control policy to mitigate disease spread effectively and the value of isolation or molecular-based methodologies.

7.4 Conclusions

Brucella spp. poses significant challenges to the dairy industry in China, affecting both the health of dairy cattle and the financial viability of dairy herds. To address this disease cost-effectively, the studies in this thesis have evaluated the four serological tests to detect antibodies against *Brucella* spp., and explored farm biosecurity practices among Henan dairy farmers. Furthermore, the direct effects of this disease on dairy cow productivity were quantified and offered for subsequent financial analyses. Vaccination was demonstrated to be the most economically favorable approach to control *Brucella* spp. for Henan dairy herds with an advantageous benefit-cost ratio and net present value. This thesis contributes substantially to knowledge of the epidemiology of *Brucella* spp. in dairy cattle and offers economic solutions and strategic insights aimed at controlling *Brucella* spp. in dairy herds in Henan Province, China. The methodologies and findings presented here may not only help in mitigating the current impacts of the disease but also serve as a foundation for future research initiatives aimed at eliminating brucellosis from the dairy sector in China.

Bibliography

Adlam, G. H. (1978). The eradication of bovine brucellosis in New Zealand: History and objectives. *New Zealand Veterinary Journal*, *26*(3), 42-43. https://doi.org/10.1080/00480169.1978.34492

Alamian, S., & Dadar, M. (2020). *Brucella melitensis* infection in dog: a critical issue in the control of brucellosis in ruminant farms. *Comparative Immunology, Microbiology and Infectious Diseases*, *73*, 101554. https://doi.org/10.1016/j.cimid.2020.101554

Aleri, J. W., & Laurence, M. (2020). A description of biosecurity practices among selected dairy farmers across Australia. *Animal Production Science*, *60*(14), 1711-1720. https://doi.org/10.1071/AN19340

Álvarez, J., Sáez, J. L., García, N., Serrat, C., Pérez-Sancho, M., González, S., Ortega, M. J., Gou, J., Carbajo, L., Garrido, F., Goyache, J., & Domínguez, L. (2011). Management of an outbreak of brucellosis due to *B. melitensis* in dairy cattle in Spain. *Research in Veterinary Science*, *90*(2), 208-211. https://doi.org/10.1016/j.rvsc.2010.05.028

Alves, A. J. S., Rocha, F., Amaku, M., Ferreira, F., Telles, E. O., Grisi Filho, J. H. H., Ferreira Neto, J. S., Zylbersztajn, D., & Dias, R. A. (2015). Economic analysis of vaccination to control bovine brucellosis in the States of Sao Paulo and Mato Grosso, Brazil. *Preventive Veterinary Medicine*, *118*(4), 351-358. https://doi.org/10.1016/j.prevetmed.2014.12.010

An, C., Nie, S., Sun, Y., Fan, S., Luo, B., Li, Z., Liu, Z., & Chang, W. (2022). Seroprevalence trend of human brucellosis and MLVA genotyping characteristics of Brucella melitensis in Shaanxi Province, China, during 2008–2020. *Transboundary and Emerging Diseases*, *69*(4), e423-e434. https://doi.org/10.1111/tbed.14320

Ana Cláudia, C., Juan García, D., & Adosinda Maria, C. (2015). Risk Factors for *Brucella* spp. in Domestic and Wild Animals. In B. Manal Mohammad (Ed.), *Updates on Brucellosis* (pp. Ch. 1). IntechOpen. https://doi.org/10.5772/61325

Anyanwu, U., Dimov, A., Zinsstag, J., Tediosi, F., & Markosyan, T. (2024). Profitability and cost-effectiveness analysis of brucellosis control in Armenia: A One Health approach. *CABI One Health*, *3*(1).

Arif, S., Heller, J., Hernandez-Jover, M., McGill, D. M., & Thomson, P. C. (2018). Evaluation of three serological tests for diagnosis of bovine brucellosis in smallholder farms in Pakistan by estimating sensitivity and specificity using Bayesian latent class analysis. *Preventive Veterinary Medicine*, *149*, 21-28. https://doi.org/10.1016/j.prevetmed.2017.11.002

Baldi, P. C., Giambartolomei, G. H., Goldbaum, F. A., Abdón, L. F., Velikovsky, C. A., Kittelberger, R., & Fossati, C. A. (1996). Humoral immune response against lipopolysaccharide and cytoplasmic proteins of Brucella abortus in cattle vaccinated with B. abortus S19 or experimentally infected with Yersinia enterocolitica serotype 0:9. *Clinical Diagnostic Laboratory Immunology*, *3*(4), 472-476. https://doi.org/doi:10.1128/cdli.3.4.472-476.1996

Baoshan, L., Yinbo, Y., Jingbo, Z., Yi, Z., Jianghua, Y., Dawei, C., Chi, M., Donghai, Y., Bohan, Y., Rongnian, Z., Sheng, F., Jun, Z., Han, X., & Chen, Z. (2021). Combined nucleic acid assays for diagnosis of A19 vaccine-caused human brucellosis. *Transboundary and Emerging Diseases*, *68*(2), 368-374. https://doi.org/10.1111/tbed.13685

Barrington, G. M., Gay, J. M., & Evermann, J. F. (2002). Biosecurity for neonatal gastrointestinal diseases. *Veterinary Clinics of North America: Food Animal Practice*, *18*(1), 7-34. https://doi.org/10.1016/S0749-0720(02)00005-1

Barros, M. L., Barddal, J. E. I., Santos, J. C. Q., Negreiros, R. L., Rosa, B. M., Teixeira, R. C., Prada, J. R. R., Gonçalves, V. S. P., & Ferreira Neto, J. S. (2023). Retrospective benefit-cost analysis of bovine brucellosis control in the state of Mato Grosso, Brazil. *Preventive Veterinary Medicine*, *218*, 105992. https://doi.org/10.1016/j.prevetmed.2023.105992

Bercovich, Z. (1998). Maintenance of *Brucella Abortus*-free herds: A review with emphasis on the epidemiology and the problems in diagnosing brucellosis in areas of low prevalence. *Veterinary Quarterly*, *20*(3), 81-88. https://doi.org/10.1080/01652176.1998.9694845

Bernués, A., Manrique, E., & Maza, M. T. (1997). Economic evaluation of bovine brucellosis and tuberculosis eradication programmes in a mountain area of Spain. *Preventive Veterinary Medicine*, *30*(2), 137-149. https://doi.org/10.1016/S0167-5877(96)01103-8

Blasco, J. M., Moreno, E., & Moriyón, I. (2021). Brucellosis. In *Veterinary Vaccines* (pp. 295-316). https://doi.org/10.1002/9781119506287.ch22

Bodenham, R. F., Mazeri, S., Cleaveland, S., Crump, J. A., Fasina, F. O., de Glanville, W. A., Haydon, D. T., Kazwala, R. R., Kibona, T. J., Maro, V. P., Maze, M. J., Mmbaga, B. T., Mtui-Malamsha, N. J., Shirima, G. M., Swai, E. S., Thomas, K. M., Bronsvoort, B. M. d., & Halliday, J. E. B. (2021). Latent class evaluation of the performance of serological tests for exposure to Brucella spp. in cattle, sheep, and goats in Tanzania. *PLOS Neglected Tropical Diseases*, *15*(8), e0009630. https://doi.org/10.1371/journal.pntd.0009630

Bonestroo, J., van der Voort, M., Fall, N., Emanuelson, U., Klaas, I. C., & Hogeveen, H. (2022). Estimating the nonlinear association of online somatic cell count, lactate dehydrogenase, and electrical conductivity with milk yield. *Journal of Dairy Science*, *105*(4), 3518-3529. https://doi.org/10.3168/jds.2021-21351

Branscum, A. J., Gardner, I. A., & Johnson, W. O. (2004). Bayesian modeling of animal- and herd-level prevalences. *Preventive Veterinary Medicine*, *66*(1), 101-112. https://doi.org/10.1016/j.prevetmed.2004.09.009

Brooks, S. P., & Gelman, A. (1998). General Methods for Monitoring Convergence of Iterative

Simulations. *Journal of Computational and Graphical Statistics*, *7*(4), 434-455. https://doi.org/10.1080/10618600.1998.10474787

Buttigieg, S. C., Savic, S., Cauchi, D., Lautier, E., Canali, M., & Aragrande, M. (2018). Brucellosis Control in Malta and Serbia: A One Health Evaluation. *Frontiers in Veterinary Science*, *5*. https://www.frontiersin.org/journals/veterinary-science/articles/10.3389/fvets.2018.00147

Capparelli, R., Parlato, M., Iannaccone, M., Roperto, S., Marabelli, R., Roperto, F., & Iannelli, D. (2009). Heterogeneous shedding of Brucella abortus in milk and its effect on the control of animal brucellosis. *Journal of Applied Microbiology*, *106*(6), 2041-2047. https://doi.org/10.1111/j.1365-2672.2009.04177.x

Cárdenas, L., Peña, M., Melo, O., & Casal, J. (2019). Risk factors for new bovine brucellosis infections in Colombian herds. *BMC Veterinary Research*, *15*(1), 81. https://doi.org/10.1186/s12917-019-1825-9

Chao, J., Peng, Q., Zhao, J., Zhu, X., Ruan, J., Lu, S., Hu, R., Li, J., Chen, X., Chen, H., Fu, Z. F., Zhao, L., Zhou, M., & Guo, A. (2021). Different rabies outbreaks on two beef cattle farms in the same province of China: Diagnosis, virus characterization and epidemiological analysis. *Transboundary and Emerging Diseases*, *68*(3), 1216-1228. https://doi.org/10.1111/tbed.13775

Charrad, M., Ghazzali, N., Boiteau, V., & Niknafs, A. (2014). NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set. *Journal of Statistical Software*, *61*(6), 1 - 36. https://doi.org/10.18637/jss.v061.i06

Chen, D., Zhou, M., Tang, C., Yang, A., Chen, B., Chen, D., Guo, L., Liang, L., Hou, W., Wen, H., Mo, X., Zhang, L., & Yin, N. (2019). A seroepidemiological survey of bovine brucellosis in Sichuan Province from 2015 to 2017. *Chinese Journal of Veterinary Medicine*, *55*(12), 66-67.

Chen, Y., Guo, A., Hu, C., Chen, X., Chen, H., Cao, Y., & Cao, W. (2020). *Biosecurity technical guideline for large-scale cattle farms - DB42/T 1585-2020*. National Public Service Platform for Standards Information. https://std.samr.gov.cn/

Chen, Y., Wang, Y., Robertson, I. D., Hu, C., Chen, H., & Guo, A. (2021). Key issues affecting the current status of infectious diseases in Chinese cattle farms and their control through vaccination. *Vaccine*, *39*(30), 4184-4189. https://doi.org/10.1016/j.vaccine.2021.05.078

Cheng, J., & Ward, M. P. (2022). Risk factors for the spread of African Swine Fever in China: A systematic review of Chinese-language literature. *Transboundary and Emerging Diseases*, *69*(5), e1289-e1298. https://doi.org/10.1111/tbed.14573

Cheung, A., Dufour, S., Jones, G., Kostoulas, P., Stevenson, M. A., Singanallur, N. B., & Firestone, S. M. (2021). Bayesian latent class analysis when the reference test is imperfect. *Rev Sci Tech*, *40*(1), 271-286. https://doi.org/10.20506/rst.40.1.3224

China Animal Disease Prevention and Control Center. (2023). *Technical guideline for evaluation of animal disease eradication in farms*. Retrieved from http://nynct.gxzf.gov.cn/ykzxzl/jkjcjs/P020230605363165888025.pdf

China Dairy Association. (2022). *Statistical Summary of Dairy Industry in China*. https://www.dac.org.cn/

China International Import Expo Exhibitors Alliance Dairy Committee. (2021). *China's dairy product imports report*. http://images.mofcom.gov.cn/perth/202203/20220301120323995.pdf

Choi, Y.-K., Johnson, W. O., Collins, M. T., & Gardner, I. A. (2006). Bayesian inferences for receiver operating characteristic curves in the absence of a gold standard. *Journal of Agricultural, Biological, and Environmental Statistics*, *11*(2), 210. https://doi.org/10.1198/108571106X110883

Christensen, J., & Gardner, I. A. (2000). Herd-level interpretation of test results for epidemiologic studies of animal diseases. *Preventive Veterinary Medicine*, *45*(1), 83-106. https://doi.org/https://doi.org/10.1016/S0167-5877(00)00118-5

Coupe, A. (2021). *Investigating Toxoplasma gondii in the marine environment in New Zealand: from cats to kai moana (shellfish) : a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Veterinary Science at Massey University, School of Veterinary Science, Massey University, Palmerston North, New Zealand* [Doctoral, Massey University]. http://hdl.handle.net/10179/16547

Cui, B., Liu, Z. P., Ke, J., & Tian, Y. (2019). Determinants of highly pathogenic avian influenza outbreak information sources, risk perception and adoption of biosecurity behaviors among poultry farmers in China. *Preventive Veterinary Medicine*, *167*, 25-31. https://doi.org/10.1016/j.prevetmed.2019.03.018

Cui, J., Wang, J., & Gu, Z. (2014). Investigation of Prevalence and Risk Factors of Brucellosis in Dairy Cows in X City of Liaoning Province. *China Animal Health Inspection*, *31*(11), 51-54.

Dadar, M., Fakhri, Y., Shahali, Y., & Mousavi Khaneghah, A. (2020). Contamination of milk and dairy products by Brucella species: A global systematic review and meta-analysis. *Food Research International*, *128*, 108775. https://doi.org/https://doi.org/10.1016/j.foodres.2019.108775

Dajer, A., Luna-Martínez, E., Zapata, D., Villegas, S., Gutiérrez, E., Peña, G., Gurría, F., Nielsen, K., & Gall, D. (1999). Evaluation of a fluorescence-polarization assay for the diagnosis of bovine brucellosis in México. *Preventive Veterinary Medicine*, *40*(1), 67-73. https://doi.org/10.1016/S0167-5877(99)00004-5

Davidson, R. M. (2002). Control and eradication of animal diseases in New Zealand. *New Zealand Veterinary Journal*, *50*(sup3), 6-12. https://doi.org/10.1080/00480169.2002.36259 de Alencar Mota, A. L. A., Ferreira, F., Ferreira Neto, J. S., Dias, R. A., Amaku, M., Hildebrand Grisi-Filho, J. H., Telles, E. O., & Picão Gonçalves, V. S. (2016). Large-scale study of herd-level risk factors for bovine brucellosis in Brazil. *Acta Tropica*, *164*, 226-232. https://doi.org/10.1016/j.actatropica.2016.09.016

de Oliveira, M. M., Pereira, C. R., de Oliveira, I. R. C., Godfroid, J., Lage, A. P., & Dorneles, E. M. S. (2022). Efficacy of Brucella abortus S19 and RB51 vaccine strains: a systematic review and meta-analysis. *Transboundary and Emerging Diseases*, *69*, e32-e51. https://doi.org/10.1111/tbed.14259

Dean, A. S., Crump, L., Greter, H., Hattendorf, J., Schelling, E., & Zinsstag, J. (2012a). Clinical Manifestations of Human Brucellosis: A Systematic Review and Meta-Analysis. *PLOS Neglected Tropical Diseases*, *6*(12), e1929. https://doi.org/10.1371/journal.pntd.0001929

Dean, A. S., Crump, L., Greter, H., Schelling, E., & Zinsstag, J. (2012b). Global Burden of Human Brucellosis: A Systematic Review of Disease Frequency. *PLOS Neglected Tropical Diseases*, *6*(10), e1865. https://doi.org/10.1371/journal.pntd.0001865

Deb Nath, N., Ahmed, S. S. U., Malakar, V., Hussain, T., Chandra Deb, L., & Paul, S. (2023). Sero-prevalence and risk factors associated with brucellosis in dairy cattle of Sylhet District, Bangladesh: A cross-sectional study. *Veterinary Medicine and Science*, *9*(3), 1349-1358. https://doi.org/https://doi.org/10.1002/vms3.1100

Dendukuri, N., Bélisle, P., & Joseph, L. (2010). Bayesian sample size for diagnostic test studies in the absence of a gold standard: Comparing identifiable with non-identifiable models. *Statistics in Medicine*, *29*(26), 2688-2697. https://doi.org/10.1002/sim.4037

Dendukuri, N., Rahme, E., Bélisle, P., & Joseph, L. (2004). Bayesian Sample Size Determination for Prevalence and Diagnostic Test Studies in the Absence of a Gold Standard Test. *Biometrics*, *60*(2), 388-397. https://doi.org/10.1111/j.0006-341X.2004.00183.x

Deng, M., Chen, N., Guidarini, C., Xu, Z., Zhang, J., Cai, L., Yuan, S., Sun, Y., & Metcalfe, L. (2020). Prevalence and genetic diversity of bovine viral diarrhea virus in dairy herds of China. *Veterinary Microbiology*, *242*, 108565. https://doi.org/10.1016/j.vetmic.2019.108565

Deng, M., Ji, S., Fei, W., Raza, S., He, C., Chen, Y., Chen, H., & Guo, A. (2015). Prevalence Study and Genetic Typing of Bovine Viral Diarrhea Virus (BVDV) in Four Bovine Species in China. *PLOS ONE*, *10*(4), e0121718. https://doi.org/10.1371/journal.pone.0121718

Denis-Robichaud, J., Kelton, D. F., Bauman, C. A., Barkema, H. W., Keefe, G. P., & Dubuc, J. (2019a). Biosecurity and herd health management practices on Canadian dairy farms. *Journal of Dairy Science*, *102*(10), 9536-9547. https://doi.org/10.3168/jds.2018-15921

Denis-Robichaud, J., Kelton, D. F., Bauman, C. A., Barkema, H. W., Keefe, G. P., & Dubuc, J.

(2019b). Canadian dairy farmers' perception of the efficacy of biosecurity practices. *Journal of Dairy Science*, *102*(11), 10657-10669. https://doi.org/10.3168/jds.2019-16312

Department of Agriculture and Rural Affairs of Henan Province. (2022a). *Five-Year Action Plan for the Prevention and Control of Brucellosis in Livestock in Henan Province (2022-2026)*. https://nynct.henan.gov.cn/2022/06-10/2465190.html

Department of Agriculture and Rural Affairs of Henan Province. (2022b). *Henan Province Animal Disease Compulsory Immunization Subsidy Policy Reform Implementation Plan*. https://nynct.henan.gov.cn/2022/01-21/2386193.html

Department of Agriculture and Rural Affairs of Henan Province. (2022c). *Henan Province poultry and livestock market prices*. https://nynct.henan.gov.cn/qcjgxq/

Deqiu, S., Donglou, X., & Jiming, Y. (2002). Epidemiology and control of brucellosis in China. *Veterinary Microbiology*, *90*(1), 165-182. https://doi.org/10.1016/S0378-1135(02)00252-3

Dhand, N. K., Singh, J., Josan, H. S., Singh, B. B., Jaswal, N., Tiwari, H. K., Kostoulas, P., Khatkar, M. S., Aulakh, R. S., Kaur, M., & Gill, J. P. S. (2021). The feasibility and acceptability of various bovine brucellosis control strategies in India. *Preventive Veterinary Medicine*, *189*, 105291. https://doi.org/10.1016/j.prevetmed.2021.105291

Dieste-Pérez, L., Frankena, K., Blasco, J. M., Muñoz, P. M., & de Jong, M. C. M. (2016). Efficacy of antibiotic treatment and test-based culling strategies for eradicating brucellosis in commercial swine herds. *Preventive Veterinary Medicine*, *126*, 105-110. https://doi.org/10.1016/j.prevetmed.2016.01.033

Dijkhuizen, A. A., Huirne, R. B. M., & Jalvingh, A. W. (1995). Economic analysis of animal diseases and their control. *Preventive Veterinary Medicine*, *25*(2), 135-149. https://doi.org/10.1016/0167-5877(95)00535-8

Dohoo, I. R., Martin, W., & Stryhn, H. E. (2009). *Veterinary Epidemiologic Research*. University of Prince Edward Island.

Dong, S.-B., Xiao, D., Liu, J.-Y., Bi, H.-M., Zheng, Z.-R., Wang, L.-D., Yang, X.-W., Tian, G.-Z., Zhao, H.-Y., Piao, D.-R., Xing, Z.-F., & Jiang, H. (2021). Fluorescence polarization assay improves the rapid detection of human brucellosis in China. *Infectious Diseases of Poverty*, *10*(1), 46. https://doi.org/10.1186/s40249-021-00834-3

Ducrotoy, M. J., Conde-Álvarez, R., Blasco, J. M., & Moriyón, I. (2016). A review of the basis of the immunological diagnosis of ruminant brucellosis. *Veterinary Immunology and Immunopathology*, *171*, 81-102. https://doi.org/10.1016/j.vetimm.2016.02.002

Ducrotoy, M. J., Muñoz, P. M., Conde-Álvarez, R., Blasco, J. M., & Moriyón, I. (2018). A systematic review of current immunological tests for the diagnosis of cattle brucellosis. *Preventive Veterinary Medicine*, *151*, 57-72.

https://doi.org/10.1016/j.prevetmed.2018.01.005

England, T., Kelly, L., Jones, R. D., MacMillan, A., & Wooldridge, M. (2004). A simulation model of brucellosis spread in British cattle under several testing regimes. *Preventive Veterinary Medicine*, *63*(1), 63-73. https://doi.org/10.1016/j.prevetmed.2004.01.009

Etefa, M., Kabeta, T., Merga, D., & Debelo, M. (2022). Cross-Sectional Study of Seroprevalence and Associated Risk Factors of Bovine Brucellosis in Selected Districts of Jimma Zone, South Western Oromia, Ethiopia. *BioMed Research International*, *2022*(1), 9549942. https://doi.org/https://doi.org/10.1155/2022/9549942

Eurofins-INGENASA. (2022). *INgezim® BRUCELLA Compac 2.0 Detection of specific antibodies to LPS of Brucella spp., in bovine, ovine, caprine and porcine sera samples.* Retrieved 06-15 from https://ingenasa.eurofins-technologies.com/media/10747/ft-ei-10bruk3-ingezim-Brucella-compac-2-0_200721.pdf

FAO. (2023). *Data on Per capita consumption of milk, excluding butter*. Our World in Data. https://ourworldindata.org/grapher/per-capita-milk-consumption

Ferreira, B. F. S., Barros, M. L., Ferreira, F., Rocha, A., Dias, R. A., Filho, J. H. H. G., Heinemann, M. B., Telles, E. O., Alevate, G. C., & Neto, J. S. F. (2023). Economic analysis of bovine brucellosis control in the Rondônia state, Brazil. *Tropical Animal Health and Production*, *55*(3), 225. https://doi.org/10.1007/s11250-023-03635-y

Fountain, J., Brookes, V., Kirkeby, C., Manyweathers, J., Maru, Y., & Hernandez-Jover, M. (2022). One size does not fit all: Exploring the economic and non-economic outcomes of on-farm biosecurity for bovine viral diarrhoea virus in Australian beef production. *Preventive Veterinary Medicine*, *208*, 105758. https://doi.org/10.1016/j.prevetmed.2022.105758

Franco, M. P., Mulder, M., Gilman, R. H., & Smits, H. L. (2007). Human brucellosis. *The Lancet Infectious Diseases*, *7*(12), 775-786. https://doi.org/10.1016/S1473-3099(07)70286-4

Fu, Y., Wang, S., Liu, W., Lin, Y., Li, X., & Qi, Q. (2012). Surveillance and analysis of brucellosis in cattle and sheep in Qinghai Province from 2007 to 2011. *China Herbivore Science*, *32*(03), 50-51.

Gall, D., & Nielsen, K. (2004). Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Rev Sci Tech*, *23*(3), 989-1002.

Gardner, I. A., Colling, A., & Greiner, M. (2019). Design, statistical analysis and reporting standards for test accuracy studies for infectious diseases in animals: Progress, challenges and recommendations. *Preventive Veterinary Medicine*, *162*, 46-55. https://doi.org/10.1016/j.prevetmed.2018.10.023

Gardner, I. A., Greiner, M., & Dubey, J. P. (2010). Statistical Evaluation of Test Accuracy Studies for Toxoplasma gondii in Food Animal Intermediate Hosts. *Zoonoses and*

Public Health, *57*(1), 82-94. https://doi.org/10.1111/j.1863-2378.2009.01281.x

General Office of Henan Provincial Government. (2022). *Beef and Dairy Cattle Industry Development Action Plan in Henan Province*. https://nynct.henan.gov.cn/2022/04-13/2430529.html

Geng, H. (2022). *Establishment of Indirect ELISA Method for Brucella VirB9 Protein and Epidemiological Investigation of Bovine Brucellosis in Shandong Province in 2021* [Master, Shandong Agricultural University].

Georgiadis, M. P., Johnson, W. O., Gardner, I. A., & Singh, R. (2003). Correlation-Adjusted Estimation of Sensitivity and Specificity of Two Diagnostic Tests. *Journal of the Royal Statistical Society Series C: Applied Statistics*, *52*(1), 63-76. https://doi.org/10.1111/1467-9876.00389

Gilbert, W. H., HÄSler, B. N., & Rushton, J. (2014). Influences of farmer and veterinarian behaviour on emerging disease surveillance in England and Wales. *Epidemiology and Infection*, *142*(1), 172-186. https://doi.org/10.1017/S0950268813000484

Godfroid, J., Garin-Bastuji, B., Saegerman, C., & Blasco, J. M. (2013). Brucellosis in terrestrial wildlife. *Rev Sci Tech*, *32*(1), 27-42. https://doi.org/10.20506/rst.32.1.2180

Godfroid, J., Scholz, H. C., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., Whatmore, A. M., Cloeckaert, A., Blasco, J. M., Moriyon, I., Saegerman, C., Muma, J. B., Al Dahouk, S., Neubauer, H., & Letesson, J. J. (2011). Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive Veterinary Medicine*, *102*(2), 118-131. https://doi.org/10.1016/j.prevetmed.2011.04.007

Greening, S. S., Mulqueen, K., Rawdon, T. G., French, N. P., & Gates, M. C. (2020). Estimating the level of disease risk and biosecurity on commercial poultry farms in New Zealand. *New Zealand Veterinary Journal*, *68*(5), 261-271. https://doi.org/10.1080/00480169.2020.1746208

Greiner, M., & Gardner, I. A. (2000a). Application of diagnostic tests in veterinary epidemiologic studies. *Preventive Veterinary Medicine*, *45*(1), 43-59. https://doi.org/10.1016/S0167-5877(00)00116-1

Greiner, M., & Gardner, I. A. (2000b). Epidemiologic issues in the validation of veterinary diagnostic tests. *Preventive Veterinary Medicine*, *45*(1), 3-22. https://doi.org/10.1016/S0167-5877(00)00114-8

Greiner, M., Pfeiffer, D., & Smith, R. D. (2000c). Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Preventive Veterinary Medicine*, *45*(1), 23-41. https://doi.org/10.1016/S0167-5877(00)00115-X

Guinat, C., Vergne, T., Kocher, A., Chakraborty, D., Paul, M. C., Ducatez, M., & Stadler, T. (2021). What can phylodynamics bring to animal health research? *Trends in Ecology & Evolution*, *36*(9), 837-847. https://doi.org/10.1016/j.tree.2021.04.013

Guo, R., Chen, F., Mei, C., Dai, Z., Yan, L., & Shi, Z. (2020). Conception Rate and Reproductive Hormone Secretion in Holstein Cows Immunized against Inhibin and Subjected to the Ovsynch Protocol. *Animals*, *10*(2).

Hadrich, J. C., Wolf, C. A., Lombard, J., & Dolak, T. M. (2018). Estimating milk yield and value losses from increased somatic cell count on US dairy farms. *Journal of Dairy Science*, *101*(4), 3588-3596. https://doi.org/10.3168/jds.2017-13840

Hagnestam-Nielsen, C., Emanuelson, U., Berglund, B., & Strandberg, E. (2009). Relationship between somatic cell count and milk yield in different stages of lactation. *Journal of Dairy Science*, *92*(7), 3124-3133. https://doi.org/10.3168/jds.2008-1719

Han, J.-H., Weston, J. F., Heuer, C., & Gates, M. C. (2020). Modelling the economics of bovine viral diarrhoea virus control in pastoral dairy and beef cattle herds. *Preventive Veterinary Medicine*, *182*, 105092. https://doi.org/10.1016/j.prevetmed.2020.105092

Han, T., Liu, J., Liu, W., Zeng, Z., He, F., Xiao, F., & Yanqin, D. (2022). Epidemiological characteristics and control strategy of human brucellosis in Fujian, 2016-2020. *Disease Surveillance*, *37*(11), 1442-1446.

Hand, K. J., Godkin, A., & Kelton, D. F. (2012). Milk production and somatic cell counts: A cow-level analysis. *Journal of Dairy Science*, *95*(3), 1358-1362. https://doi.org/10.3168/jds.2011-4927

Hao, Z., Xing, H., Zhang, J., Wang, L., Kong, L., Ying, H., Wei, J., Kong, M., Ma, X., Pan, Z., & Liu, S. (2014). Surveillance and elimination of tuberculosis and brucellosis in cattle and sheep in Qufu city. *Shandong Journal of Animal Science and Veterinary Medicine*, *35*(03), 43-45.

Hegazy, Y. M., Ridler, A. L., & Guitian, F. J. (2009). Assessment and simulation of the implementation of brucellosis control programme in an endemic area of the Middle East. *Epidemiology and Infection*, *137*(10), 1436-1448. https://doi.org/10.1017/S0950268809002301

Henan DHI. (2023). *Annual report on the milk production and DHI measurement of dairy industry in Henan Province*. http://www.hndhi.com/

Henan Government Procurement Centre. (2022). *Brucellosis Vaccines Procurement*. https://www.hngp.gov.cn/henan/content?infoId=1526822

Herrera, E., Palomares, G., & Díaz-Aparicio, E. (2008). Milk Production Increase in a Dairy Farm under a Six-Year Brucellosis Control Program. *Annals of the New York Academy of Sciences*, *1149*(1), 296-299. https://doi.org/10.1196/annals.1428.011

Higgins, J. L., Gonzalez-Juarrero, M., & Bowen, R. A. (2017). Evaluation of shedding, tissue burdens, and humoral immune response in goats after experimental challenge with the virulent Brucella melitensis strain 16M and the reduced virulence vaccine strain Rev. 1. *PLOS ONE*, *12*(10), e0185823. https://doi.org/10.1371/journal.pone.0185823

Hill, E. M., Prosser, N. S., Brown, P. E., Ferguson, E., Green, M. J., Kaler, J., Keeling, M. J., & Tildesley, M. J. (2023). Incorporating heterogeneity in farmer disease control behaviour into a livestock disease transmission model. *Preventive Veterinary Medicine*, *219*, 106019. https://doi.org/10.1016/j.prevetmed.2023.106019

Højsgaard, S., Halekoh, U., & Yan, J. (2006). The R package geepack for generalized estimating equations. *Journal of Statistical Software*, *15*, 1-11.

Holt, H. R., Bedi, J. S., Kaur, P., Mangtani, P., Sharma, N. S., Gill, J. P. S., Singh, Y., Kumar, R., Kaur, M., McGiven, J., & Guitian, J. (2021). Epidemiology of brucellosis in cattle and dairy farmers of rural Ludhiana, Punjab. *PLOS Neglected Tropical Diseases*, *15*(3), e0009102. https://doi.org/10.1371/journal.pntd.0009102

Holt, H. R., Eltholth, M. M., Hegazy, Y. M., El-Tras, W. F., Tayel, A. A., & Guitian, J. (2011). Brucella spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). *BMC Public Health*, *11*(1), 341. https://doi.org/10.1186/1471-2458-11-341

Holt, H. R., Walker, M., Beauvais, W., Kaur, P., Bedi, J. S., Mangtani, P., Sharma, N. S., Gill, J. P. S., Godfroid, J., McGiven, J., & Guitian, J. (2023). Modelling the control of bovine brucellosis in India. *Journal of The Royal Society Interface*, *20*(200), 20220756. https://doi.org/10.1098/rsif.2022.0756

Huang, C.-H., Furukawa, K., & Kusaba, N. (2023). Estimating the nonlinear interaction between somatic cell score and differential somatic cell count on milk production by parity using generalized additive models. *Journal of Dairy Science*, *106*(11), 7942-7953. https://doi.org/10.3168/jds.2022-22958

Huang, C. (2019). *Epidemiological Investigation and Pathogen Isolation and Biotype Identification of Brucellosis in Some Large-scale Cattle Farms in Shihezi City* [Master, Shihezi University].

Huang, D. (2012). *Research on Epidemiological Survey of Cow Brucellosis in Changping District, Beijing from 2000 to 2009* [Master, Chinese Academy of Agricultural Sciences].

Hugh-Jones, M. E., Ellis, P. R., & Felton, M. R. (1975). *An assessment of the eradication of bovine brucellosis in England and Wales*.

Husson, F., Lê, S., & Pagès, J. (2017). *Exploratory Multivariate Analysis by Example Using R (2nd ed.)*. Chapman and Hall/CRC. https://doi.org/10.1201/b21874

Janke, N. R., Williamson, C. H. D., Drees, K. P., Suárez-Esquivel, M., Allen, A. R., Ladner, J. T., Quance, C. R., Robbe-Austerman, S., O'Callaghan, D., Whatmore, A. M., & Foster, J. T. (2023). Global phylogenomic diversity of Brucella abortus: spread of a dominant lineage. *Frontiers in Microbiology*, *14*. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2023.1287046

Jiang, J., Dai, G., Wei, B., Xie, L., Liang, Z., Li, M., & Liu, J. (2014). Epidemiological survey of major diseases of dairy cattle in Wuzhou from 2003 to 2013. *Journal of Animal Science and Veterinary Medicine*, *33*(05), 99-101.

Johnson, W. O., Jones, G., & Gardner, I. A. (2019). Gold standards are out and Bayes is in: Implementing the cure for imperfect reference tests in diagnostic accuracy studies. *Preventive Veterinary Medicine*, *167*, 113-127. https://doi.org/10.1016/j.prevetmed.2019.01.010

Jones, G., Johnson, W. O., Hanson, T. E., & Christensen, R. (2010). Identifiability of Models for Multiple Diagnostic Testing in the Absence of a Gold Standard. *Biometrics*, *66*(3), 855-863. https://doi.org/10.1111/j.1541-0420.2009.01330.x

Jones, G., Johnson, W. O., & Vink, W. D. (2009). Evaluating a continuous biomarker for infection by using observed disease status with covariate effects on disease. *Journal of the Royal Statistical Society: Series C (Applied Statistics)*, *58*(5), 705-717. https://doi.org/10.1111/j.1467-9876.2009.00681.x

Kamath, P. L., Foster, J. T., Drees, K. P., Luikart, G., Quance, C., Anderson, N. J., Clarke, P. R., Cole, E. K., Drew, M. L., Edwards, W. H., Rhyan, J. C., Treanor, J. J., Wallen, R. L., White, P. J., Robbe-Austerman, S., & Cross, P. C. (2016). Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. *Nature Communications*, *7*(1), 11448. https://doi.org/10.1038/ncomms11448

Kang, B. (2014). Serological investigation and analysis of zoonotic brucellosis in Wuyuan County. *Journal of Inner Mongolia Medical University*, *36*(S1), 116-117.

Kassambara, A., & Mundt, F. (2020). factoextra: Extract and Visualize the Results of Multivariate Data Analyses. *R package version 1.0.7*. https://CRAN.R-project.org/package=factoextra

Keshavarzi, H., Sadeghi-Sefidmazgi, A., Kristensen, A. R., & Stygar, A. H. (2017). Abortion studies in Iranian dairy herds: I. Risk factors for abortion. *Livestock Science*, *195*, 45-52. https://doi.org/10.1016/j.livsci.2016.11.004

Khurana, S. K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M. Z., Chhabra, R., Karthik, K., Patel, S. K., Pathak, M., Iqbal Yatoo, M., Gupta, V. K., Dhama, K., Sah, R., & Chaicumpa, W. (2021). Bovine brucellosis – a comprehensive review. *Veterinary Quarterly*, *41*(1), 61-88. https://doi.org/10.1080/01652176.2020.1868616

Kiambi, S. G., Fèvre, E. M., Omolo, J., Oundo, J., & de Glanville, W. A. (2020). Risk factors for acute human brucellosis in Ijara, north-eastern Kenya. *PLOS Neglected Tropical Diseases*, *14*(4), e0008108-e0008108. https://doi.org/10.1371/journal.pntd.0008108

Kiiza, D., Denagamage, T., Serra, R., Maunsell, F., Kiker, G., Benavides, B., & Hernandez, J. A. (2023). A systematic review of economic assessments for brucellosis control interventions in livestock populations. *Preventive Veterinary Medicine*, *213*, 105878. https://doi.org/10.1016/j.prevetmed.2023.105878

Kostoulas, P., Leontides, L., Billinis, C., & Florou, M. (2006). Application of a semi-dependent latent model in the Bayesian estimation of the sensitivity and specificity of two faecal culture methods for diagnosis of paratuberculosis in sub-clinically infected Greek dairy sheep and goats. *Preventive Veterinary Medicine*, *76*(1), 121-134. https://doi.org/10.1016/j.prevetmed.2006.04.008

Kostoulas, P., Nielsen, S. S., Branscum, A. J., Johnson, W. O., Dendukuri, N., Dhand, N. K., Toft, N., & Gardner, I. A. (2017). STARD-BLCM: Standards for the Reporting of Diagnostic accuracy studies that use Bayesian Latent Class Models. *Preventive Veterinary Medicine*, *138*, 37-47. https://doi.org/10.1016/j.prevetmed.2017.01.006

Kristensen, E., & Jakobsen, E. B. (2011). Danish dairy farmers' perception of biosecurity. *Preventive Veterinary Medicine*, *99*(2), 122-129. https://doi.org/10.1016/j.prevetmed.2011.01.010

Lai, S., Zhou, H., Xiong, W., Yu, H., Huang, Z., Yu, J., Yin, W., Wang, L., Chen, Q., Li, Y., Mu, D., Zeng, L., Ren, X., Geng, M., Zhang, Z., Cui, B., Li, T., Wang, D., Sun, Q., Wardrop, N., Tatem, A., & Wei, S. (2017). Changing Epidemiology of Human Brucellosis, China, 1955–2014. *Emerging Infectious Disease*, *23*(2), 184. https://doi.org/10.3201/eid2302.151710

Laine, C., Johnson, V., Scott, H. M., & Arenas-Gamboa, A. (2023). Global Estimate of Human Brucellosis Incidence. *Emerging Infectious Disease journal*, *29*(9), 1789. https://doi.org/10.3201/eid2909.230052

Lambert, S., Gilot-Fromont, E., Toïgo, C., Marchand, P., Petit, E., Rossi, S., & Thébault, A. (2022). Combining seroprevalence and capture-mark-recapture data to estimate the force of infection of brucellosis in a managed population of Alpine ibex. *Epidemics*, *38*, 100542. https://doi.org/10.1016/j.epidem.2022.100542

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, *25*(1), 1 - 18. https://doi.org/10.18637/jss.v025.i01

Lehane, R. (1996). *Beating the odds in a big country: the eradication of bovine brucellosis and tuberculosis in Australia*. CSIRO publishing.

Leite, B. M., Lôbo, J. R., Mendes Ruas, J. R., Ferreira, F., Geeverghese, C., Leão Freitas, M., & Goncalves, V. S. P. (2018). Economic Analysis of the Policy for Accreditation of Dairy Farms Free of Bovine Brucellosis and Tuberculosis: Challenges for Small and Large Producers in Brazil. *Journal of Agricultural Economics*, *69*(1), 262-276. https://doi.org/10.1111/1477-9552.12227

Li, J., Yuan, M., Wang, H., & Zhou, K. (2023). Government regulations, biosecurity awareness, and farmers' adoption of biosecurity measures: Evidence from pig farmers in Sichuan Province, China. *Frontiers in Sustainable Food Systems*, *7*. https://www.frontiersin.org/articles/10.3389/fsufs.2023.1106766

Li, X. (2013). *Epidemiological Survey and Preliminary Study on the Infection Mechanism of*

Cow Brucellosis in Tongliao District of Inner Mongolia [PhD, Jilin University].

Li, Y., Huang, B., Shen, C., Cai, C., Wang, Y., Edwards, J., Zhang, G., & Robertson, I. D. (2020a). Pig trade networks through live pig markets in Guangdong Province, China. *Transboundary and Emerging Diseases*, *67*(3), 1315-1329. https://doi.org/10.1111/tbed.13472

Li, Y., Tan, D., Xue, S., Shen, C., Ning, H., Cai, C., & Liu, Z. (2021). Prevalence, distribution and risk factors for brucellosis infection in goat farms in Ningxiang, China. *BMC Veterinary Research*, *17*(1), 39. https://doi.org/10.1186/s12917-021-02743-x

Li, Z., Liu, X., Xu, C., & Tang, D. (2019). Sero-epidemiological Investigation on Brucellosis in Cows in Guizhou Province During 2015 to 2017. *China Animal Health Inspection*, *36*(04), 19-21.

Li, Z., Wang, X.-m., Zhu, X., Wang, M., Cheng, H., Li, D., & Liu, Z. G. (2020b). Molecular Characteristics of Brucella Isolates Collected From Humans in Hainan Province, China. *Frontiers in Microbiology*, *11*, 452. https://www.frontiersin.org/article/10.3389/fmicb.2020.00452

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., Clarke, M., Devereaux, P. J., Kleijnen, J., & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of Clinical Epidemiology*, *62*(10), e1-e34. https://doi.org/10.1016/j.jclinepi.2009.06.006

Limmathurotsakul, D., Jamsen, K., Arayawichanont, A., Simpson, J. A., White, L. J., Lee, S. J., Wuthiekanun, V., Chantratita, N., Cheng, A., Day, N. P. J., Verzilli, C., & Peacock, S. J. (2010). Defining the True Sensitivity of Culture for the Diagnosis of Melioidosis Using Bayesian Latent Class Models. *PLOS ONE*, *5*(8), e12485. https://doi.org/10.1371/journal.pone.0012485

Lin, S., Wang, Z., Liu, X., Yu, A., Hasan, M., Bayidawulieti, J., Aximujiang, H., Li, R., Zheng, G., Liang, X., Fan, X., Hou, B., Fan, X., Abuliti, D., Shi, L., Zhang, C., Wang, Y., Ning, P., Liu, C., Li, Z., & Wang, L. (2021). Serological Prevalence Survey Among the High-Risk Populations of Brucellosis-Endemic Areas-China, 2019−2020. *China CDC Weekly*, *3*(6). https://doi.org/10.46234/ccdcw2021.027

Lindahl, J. F., Gill, J. P., Hazarika, R. A., Fairoze, N. M., Bedi, J. S., Dohoo, I., Chauhan, A. S., Grace, D., & Kakkar, M. (2019). Risk Factors for Brucella Seroprevalence in Peri-Urban Dairy Farms in Five Indian Cities. *Tropical Medicine and Infectious Disease*, *4*(2), 70.

Liu, G. (2019). *Incidence and Control Effect of Mastitis and Quarantine Purification of Brucellosis and Tuberculosis in Large-scale Dairy Farm in Reclamation Area* [Master, Northeast Agricultural University].

Liu, T., Xue, Z., Li, L., Xu, H., Wang, L., Zhang, X., Wang, G., Ma, Y., Ji, X., Zhao, Y., & Qin,

J. (2016). Epidemiological investigation of paratuberculosis and brucellosis in dairy cows in Hebei Province. *Heilongjiang Animal Science and Veterinary Medicine*(05), 180-183.

Liu, Y., Ban, F., Hu, W., Cheng, Y., Zhang, H., Xie, C., Zhao, X., Wang, D., Li, J., Shen, C., & Yan, R. (2020a). Cross-sectional Study on the Prevalence and Risks of Spreading Brucellosis among Cow Farms in Pingdingshan City of Henan Province. *China Animal Health Inspection*, *37*(12), 22-26+38.

Liu, Z., Shi, Y., Xue, C., Yuan, M., Li, Z., & Zheng, C. (2025). Epidemiological and Spatiotemporal Clustering Analysis of Human Brucellosis—China, 2019− 2023. *China CDC Weekly*, *7*(4), 130-136.

Liu, Z., Wang, H., Wang, M., & Li, Z. (2020b). Investigation of the molecular epizootiological characteristics and tracking of the geographical origins of Brucella canis strains in China. *Transboundary and Emerging Diseases*, *67*(2), 834-843. https://doi.org/10.1111/tbed.13404

Liu, Z., Wang, M., Ta, N., Fang, M., Mi, J., Yu, R., Luo, Y., Cao, X., & Li, Z. (2020c). Seroprevalence of human brucellosis and molecular characteristics of Brucella strains in Inner Mongolia Autonomous region of China, from 2012 to 2016. *Emerging Microbes & Infections*, *9*(1), 263-274. https://doi.org/10.1080/22221751.2020.1720528

Lovera, R., Fernández, M. S., Jacob, J., Lucero, N., Morici, G., Brihuega, B., Farace, M. I., Caracostantogolo, J., & Cavia, R. (2017). Intrinsic and extrinsic factors related to pathogen infection in wild small mammals in intensive milk cattle and swine production systems. *PLOS Neglected Tropical Diseases*, *11*(6), e0005722. https://doi.org/10.1371/journal.pntd.0005722

Lu, W., He, F., & Li, S. (2014). Epidemiological survey of brucellosis in Qingyang from 2009 to 2013. *Journal of Animal Science and Veterinary Medicine*, *33*(03), 73-74+76.

Lüdecke, D. (2018). ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open Source Software*, *3*(26), 772.

Lurier, T., Rousset, E., Gasqui, P., Sala, C., Claustre, C., Abrial, D., Dufour, P., de Crémoux, R., Gache, K., Delignette-Muller, M. L., Ayral, F., & Jourdain, E. (2021). Evaluation using latent class models of the diagnostic performances of three ELISA tests commercialized for the serological diagnosis of Coxiella burnetii infection in domestic ruminants. *Veterinary Research*, *52*(1), 56. https://doi.org/10.1186/s13567-021-00926-w

Ma, Q., Shen, C., & Liu, J. (2014). Serological Investigation and Risk Analysis of Dairy Cow Brucellosis in Laixi of Qingdao. *China Animal Health Inspection*, *31*(11), 55-59.

Ma, S.-y., Liu, Z.-g., Zhu, X., Zhao, Z.-z., Guo, Z.-w., Wang, M., Cui, B.-y., Li, J.-y., & Li, Z.-j. (2020). Molecular epidemiology of Brucella abortus strains from cattle in Inner Mongolia, China. *Preventive Veterinary Medicine*, *183*, 105080. https://doi.org/10.1016/j.prevetmed.2020.105080

Mantur, B. G., Amarnath, S. K., & Shinde, R. S. (2007). REVIEW OF CLINICAL AND LABORATORY FEATURES OF HUMAN BRUCELLOSIS. *Indian Journal of Medical Microbiology*, *25*(3), 188-202. https://doi.org/10.1016/S0255-0857(21)02105-8

Marquetoux, N., Stevenson, M. A., Wilson, P., Ridler, A., & Heuer, C. (2016). Using social network analysis to inform disease control interventions. *Preventive Veterinary Medicine*, *126*, 94-104. https://doi.org/10.1016/j.prevetmed.2016.01.022

Marquetoux, N., Vignes, M., Burroughs, A., Sumner, E., Sawford, K., & Jones, G. (2023). Evaluation of the accuracy of the IDvet serological test for Mycoplasma bovis infection in cattle using latent class analysis of paired serum ELISA and quantitative real-time PCR on tonsillar swabs sampled at slaughter. *PLOS ONE*, *18*(5), e0285598. https://doi.org/10.1371/journal.pone.0285598

Marsh, W. (1999). The economics of animal health in farmed livestock at the herd level. *Revue scientifique et technique (International Office of Epizootics)*, *18*(2), 357-366.

Martin, V., Zhou, X., Marshall, E., Jia, B., Fusheng, G., FrancoDixon, M. A., DeHaan, N., Pfeiffer, D. U., Soares Magalhães, R. J., & Gilbert, M. (2011). Risk-based surveillance for avian influenza control along poultry market chains in South China: The value of social network analysis. *Preventive Veterinary Medicine*, *102*(3), 196-205. https://doi.org/10.1016/j.prevetmed.2011.07.007

Matope, G., Bhebhe, E., Muma, J. B., Oloya, J., Madekurozwa, R. L., Lund, A., & Skjerve, E. (2011). Seroprevalence of brucellosis and its associated risk factors in cattle from smallholder dairy farms in Zimbabwe. *Tropical Animal Health and Production*, *43*(5), 975-982. https://doi.org/10.1007/s11250-011-9794-4

McCarthy, M. C., O'Grady, L., McAloon, C. G., & Mee, J. F. (2021). A survey of biosecurity and health management practices on Irish dairy farms engaged in contract-rearing. *Journal of Dairy Science*, *104*(12), 12859-12870. https://doi.org/10.3168/jds.2021-20500

McDermott, J., Grace, D., & Zinsstag, J. (2013). Economics of brucellosis impact and control in low-income countries. *Rev Sci Tech*, *32*(1), 249-261.

Megahed, A., Kandeel, S., Alshaya, D. S., Attia, K. A., AlKahtani, M. D. F., Albohairy, F. M., & Selim, A. (2022). A comparison of logistic regression and classification tree to assess brucellosis associated risk factors in dairy cattle. *Preventive Veterinary Medicine*, *203*, 105664. https://doi.org/10.1016/j.prevetmed.2022.105664

Mellado, M., Garcia, A. M., Arellano-Reynoso, B., Diaz-Aparicio, E., & Garcia, J. E. (2014). Milk yield and reproductive performance of brucellosis-vaccinated but seropositive Holstein cows. *Tropical Animal Health and Production*, *46*(2), 391-397. https://doi.org/10.1007/s11250-013-0502-4

Mellado, M., Reséndiz, D., Martínez, A. M., de Santiago, M. A., Véliz, F. G., & García, J. E.

(2015). Milk yield and reproductive performance of Holstein cows testing positive for bovine tuberculosis. *Tropical Animal Health and Production*, *47*(6), 1061-1066. https://doi.org/10.1007/s11250-015-0828-1

Mellado, M., Treviño, N., Véliz, F. G., Macías-Cruz, U., Avendaño-Reyes, L., de Santiago, A., & García, J. E. (2021). Effect of co-positivity for brucellosis and tuberculosis on milk yield and fertility of Holstein cows. *Tropical Animal Health and Production*, *53*(5), 504. https://doi.org/10.1007/s11250-021-02952-4

Meng, W., Chen, P., XU, R., Liu, S., Qiu, Z., Zhu, R., & Li, Y. (2013). Epidemiological investigation of brucellosis in cattle and sheep in Shizhong District, Zaozhuang. *Shandong Journal of Animal Science and Veterinary Medicine*, *34*(10), 71-72.

Miao, F., Li, N., Yang, J., Chen, T., Liu, Y., Zhang, S., & Hu, R. (2021). Neglected challenges in the control of animal rabies in China. *One Health*, *12*, 100212. https://doi.org/10.1016/j.onehlt.2021.100212

Ministry of Agriculture and Rural Affairs. (2016). *National Brucellosis Control Plan (2016-2020)*. http://www.moa.gov.cn/gk/tzgg_1/tz/201609/t20160909_5270524.htm

Ministry of Agriculture and Rural Affairs. (2022). *Five-year action plan for animal brucellosis prevention and control (2022-2026)*. Beijing Retrieved from http://www.moa.gov.cn/govpublic/xmsyj/202203/P020220330377158279349.pdf

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L. A., & Group, P.-P. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews*, *4*(1), 1. https://doi.org/10.1186/2046-4053-4-1

Montiel, O. D., Bruce, M., Frankena, K., Udo, H., van der Zijpp, A., & Rushton, J. (2015). Financial analysis of brucellosis control for small-scale goat farming in the Bajío region, Mexico. *Preventive Veterinary Medicine*, *118*(4), 247-259. https://doi.org/10.1016/j.prevetmed.2014.11.014

Moriyón, I., Blasco, J. M., Letesson, J. J., De Massis, F., & Moreno, E. (2023). Brucellosis and One Health: Inherited and Future Challenges. *Microorganisms*, *11*(8).

Munn, Z., Peters, M. D. J., Stern, C., Tufanaru, C., McArthur, A., & Aromataris, E. (2018). Systematic review or scoping review? Guidance for authors when choosing between a systematic or scoping review approach. *BMC Medical Research Methodology*, *18*(1), 143. https://doi.org/10.1186/s12874-018-0611-x

Musallam, I., Ndour, A. P., Yempabou, D., Ngong, C.-A. C., Dzousse, M. F., Mouiche-Mouliom, M.-M., Feussom, J. M. K., Ntirandekura, J. B., Ntakirutimana, D., Fane, A., Dembele, E., Doumbia, A., Ayih-Akakpo, A. A. P.-H. S., Pato, P., Pali, M., Tapsoba, A. S. R., Compaore, G. M., Gagara, H., Garba, A. I., Chengat Prakashbabu, B., Craighead, L., Takahashi, E., McGiven, J., Nguipdop-Djomo, P., Mangtani, P., Alambédji-Bada, R., Akakpo, A. J., & Guitian, J. (2019). Brucellosis in dairy herds: A public health concern in the milk supply chains of West and Central Africa. *Acta Tropica*, *197*, 105042. https://doi.org/10.1016/j.actatropica.2019.105042

Musallam, I. I., Abo-Shehada, M., Omar, M., & Guitian, J. (2015a). Cross-sectional study of brucellosis in Jordan: Prevalence, risk factors and spatial distribution in small ruminants and cattle. *Preventive Veterinary Medicine*, *118*(4), 387-396. https://doi.org/10.1016/j.prevetmed.2014.12.020

Musallam, I. I., Abo-Shehada, M. N., & Guitian, J. (2015b). Knowledge, Attitudes, and Practices Associated with Brucellosis in Livestock Owners in Jordan. *The American Society of Tropical Medicine and Hygiene*, *93*(6), 1148-1155. https://doi.org/10.4269/ajtmh.15-0294

National Bureau of Statistics of China. (2023). *China Statistical Yearbook*. China Statistics Press. https://www.stats.gov.cn/sj/ndsj/2023/indexch.htm

National Health Commission of China. (2010). National food safety standard - Raw milk (GB 19301 - 2010). In: National Public Service Platform for Standards Information.

Neta, A. V. C., Mol, J. P. S., Xavier, M. N., Paixão, T. A., Lage, A. P., & Santos, R. L. (2010). Pathogenesis of bovine brucellosis. *The Veterinary Journal*, *184*(2), 146-155. https://doi.org/10.1016/j.tvjl.2009.04.010

Nielsen, K., Gall, D., Jolley, M., Leishman, G., Balsevicius, S., Smith, P., Nicoletti, P., & Thomas, F. (1996). A homogeneous fluorescence polarization assay for detection of antibody to Brucella abortus. *Journal of Immunological Methods*, *195*(1), 161-168. https://doi.org/10.1016/0022-1759(96)00116-0

Nielsen, K., Gall, D., Smith, P., Kelly, W., Yeo, J., Kenny, K., Heneghan, T., McNamara, S., Maher, P., O'Connor, J., Walsh, B., Carroll, J., Rojas, X., Rojas, F., Perez, B., Wulff, O., Buffoni, L., Salustio, E., Gregoret, R., Samartino, L., Dajer, A., & Luna-Martinez, E. (2001). Fluorescence polarization assay for the diagnosis of bovine brucellosis: adaptation to field use. *Veterinary Microbiology*, *80*(2), 163-170. https://doi.org/10.1016/S0378-1135(00)00386-2

Ning, C., Shuyi, G., Tao, Y., Hao, Z., & Zhang, X. (2018). Epidemiological survey of human brucellosis in Inner Mongolia, China, 2010–2014: A high risk groups-based survey. *Journal of Infection and Public Health*, *11*(1), 24-29. https://doi.org/10.1016/j.jiph.2017.02.013

Ning, P., Guo, M., Guo, K., Xu, L., Ren, M., Cheng, Y., & Zhang, Y. (2013). Identification and effect decomposition of risk factors for Brucella contamination of raw whole milk in China. *PLOS ONE*, *8*(7), e68230. https://doi.org/10.1371/journal.pone.0068230

Njeru, J., Nthiwa, D., Akoko, J., Oyas, H., & Bett, B. (2021). Incidence of Brucella infection in various livestock species raised under the pastoral production system in Isiolo County, Kenya. *BMC Veterinary Research*, *17*(1), 342. https://doi.org/10.1186/s12917-021-03036-z

Nobrega, D. B., French, J. E., & Kelton, D. F. (2023). A scoping review of the testing of bulk tank milk to detect nonbacterial pathogens or herd exposure to nonbacterial pathogens in dairy cattle. *Journal of Dairy Science*, *106*(8), 5636-5658. https://doi.org/10.3168/jds.2022-22586

O'Connor, A. M., Sargeant, J. M., Dohoo, I. R., Erb, H. N., Cevallos, M., Egger, M., Ersbøll, A. K., Martin, S. W., Nielsen, L. R., Pearl, D. L., Pfeiffer, D. U., Sanchez, J., Torrence, M. E., Vigre, H., Waldner, C., & Ward, M. P. (2016). Explanation and Elaboration Document for the STROBE-Vet Statement: Strengthening the Reporting of Observational Studies in Epidemiology—Veterinary Extension. *Journal of Veterinary Internal Medicine*, *30*(6), 1896-1928. https://doi.org/10.1111/jvim.14592

Olsen, A., Nielsen, H. V., Alban, L., Houe, H., Jensen, T. B., & Denwood, M. (2022). Determination of an optimal ELISA cut-off for the diagnosis of Toxoplasma gondii infection in pigs using Bayesian latent class modelling of data from multiple diagnostic tests. *Preventive Veterinary Medicine*, *201*, 105606. https://doi.org/10.1016/j.prevetmed.2022.105606

One Health High-Level Expert, P., Adisasmito, W. B., Almuhairi, S., Behravesh, C. B., Bilivogui, P., Bukachi, S. A., Casas, N., Cediel Becerra, N., Charron, D. F., Chaudhary, A., Ciacci Zanella, J. R., Cunningham, A. A., Dar, O., Debnath, N., Dungu, B., Farag, E., Gao, G. F., Hayman, D. T. S., Khaitsa, M., Koopmans, M. P. G., Machalaba, C., Mackenzie, J. S., Markotter, W., Mettenleiter, T. C., Morand, S., Smolenskiy, V., & Zhou, L. (2022). One Health: A new definition for a sustainable and healthy future. *PLOS Pathogens*, *18*(6), e1010537. https://doi.org/10.1371/journal.ppat.1010537

Ornelas-Eusebio, E., García-Espinosa, G., Laroucau, K., & Zanella, G. (2020). Characterization of commercial poultry farms in Mexico: Towards a better understanding of biosecurity practices and antibiotic usage patterns. *PLOS ONE*, *15*(12), e0242354. https://doi.org/10.1371/journal.pone.0242354

Pappas, G. (2022). The Lanzhou Brucella Leak: The Largest Laboratory Accident in the History of Infectious Diseases? *Clinical Infectious Diseases*, *75*(10), 1845-1847. https://doi.org/10.1093/cid/ciac463

Pappas, G., Akritidis, N., Bosilkovski, M., & Tsianos, E. (2005). Brucellosis. *New England Journal of Medicine*, *352*(22), 2325-2336. https://doi.org/10.1056/NEJMra050570

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., & Tsianos, E. V. (2006). The new global map of human brucellosis. *The Lancet Infectious Diseases*, *6*(2), 91-99. https://doi.org/10.1016/S1473-3099(06)70382-6

Pei, J., Wang, Y., Zhou, Y., Huang, T., Xie, J., Guo, A., Chen, Y., & Robertson, I. D. (2025). True prevalence and spatial distribution of antibodies to Brucella spp. in goat populations in Hubei Province, People's Republic of China: Progress toward disease elimination. *Preventive Veterinary Medicine*, *235*, 106414. https://doi.org/https://doi.org/10.1016/j.prevetmed.2024.106414

Peng, C., Li, Y.-J., Huang, D.-S., & Guan, P. (2020a). Spatial-temporal distribution of human brucellosis in mainland China from 2004 to 2017 and an analysis of social and environmental factors. *Environmental Health and Preventive Medicine*, *25*(1), 1. https://doi.org/10.1186/s12199-019-0839-z

Peng, C., Zhou, H., Guan, P., Wu, W., & Huang, D.-S. (2020b). An estimate of the incidence and quantitative risk assessment of human brucellosis in mainland China. *Transboundary and Emerging Diseases*, *67*(5), 1898-1908. https://doi.org/10.1111/tbed.13518

Pepin, K. M., Carlisle, K., Anderson, D., Baker, M. G., Chipman, R. B., Benschop, J., French, N. P., Greenhalgh, S., McDougall, S., Muellner, P., Murphy, E., O'Neale, D. R. J., Plank, M. J., & Hayman, D. T. S. (2024). Steps towards operationalizing One Health approaches. *One Health*, *18*, 100740. https://doi.org/https://doi.org/10.1016/j.onehlt.2024.100740

Piao, D., Li, Z., Yang, X., Tian, G., Zhao, H., & Jiang, H. (2020). Emerging Brucellosis Outbreaks Associated with Unpasteurized Milk in China. *China CDC Weekly*, *2*(46), 888-890.

Plummer, M. (2003). JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. Proceedings of the 3rd international workshop on distributed statistical computing,

Poudel, B. S., Spooner, P. G., & Matthews, A. (2016). Behavioural changes in marmots in relation to livestock grazing disturbance: an experimental test. *European Journal of Wildlife Research*, *62*(4), 491-495. https://doi.org/10.1007/s10344-016-1014-0

Qi, Q., & Cao, Y. (2013). Surveillance and epidemiological survey of brucellosis in cattle and sheep in Qinghai Province in 2012. *Shandong Journal of Animal Science and Veterinary Medicine*, *34*(08), 57-58.

Qi, S. (2019). Epidemiological Survey of Brucellosis in Dairy Cows in Urumqi County. *Grass-feeding Livestock (In Chinese)*(05), 33-37. https://doi.org/10.16863/j.cnki.1003-6377.2019.05.007

R Core Development Team. (2021). *R: A language and environment for statistical computing, R Foundation for Statistical Computing*. In https://www.r-project.org/

Rafati, N., Mehrabani-Yeganeh, H., & Hanson, T. E. (2010). Risk factors for abortion in dairy cows from commercial Holstein dairy herds in the Tehran region. *Preventive Veterinary Medicine*, *96*(3), 170-178. https://doi.org/10.1016/j.prevetmed.2010.05.008

Rahman, A. K. M. A., Smit, S., Devleesschauwer, B., Kostoulas, P., Abatih, E., Saegerman, C., Shamsuddin, M., Berkvens, D., Dhand, N. K., & Ward, M. P. (2019). Bayesian evaluation of three serological tests for the diagnosis of bovine brucellosis in Bangladesh. *Epidemiology and Infection*, *147*, e73, Article e73. https://doi.org/10.1017/S0950268818003503

Ran, X. (2018). *Epidemiological Survey of Brucellosis and Tuberculosis in Dairy Cattle in Gongjing District, Zigong* [Master, Sichuan Agricultural University].

Ran, X., Chen, X., Wang, M., Cheng, J., Ni, H., Zhang, X.-X., & Wen, X. (2018). Brucellosis seroprevalence in ovine and caprine flocks in China during 2000–2018: a systematic review and meta-analysis. *BMC Veterinary Research*, *14*(1), 393. https://doi.org/10.1186/s12917-018-1715-6

Ran, X., Cheng, J., Wang, M., Chen, X., Wang, H., Ge, Y., Ni, H., Zhang, X.-X., & Wen, X. (2019). Brucellosis seroprevalence in dairy cattle in China during 2008–2018: A systematic review and meta-analysis. *Acta Tropica*, *189*, 117-123. https://doi.org/10.1016/j.actatropica.2018.10.002

Ren, R. (2021). *Epidemic Situation of Main Diseases in Two Dairy Farms in Hainan* [Master, Xinjiang Agricultural University].

Ren, Y., Zhang, H., Sun, X., Li, Z., Cai, J., & Fan, W. (2016). Comparative study of FPA and ELISA method in the diagnosis of animal brucellosis. *Chinese Journal of Zoonoses*, *32*(9), 806-809.

Robertson, I. D. (2020). Disease Control, Prevention and On-Farm Biosecurity: The Role of Veterinary Epidemiology. *Engineering*, *6*(1), 20-25. https://doi.org/10.1016/j.eng.2019.10.004

Rodrigues, D. L., Marquetoux, N., Grisi Filho, J. H., & Ferreira Neto, J. S. (2023). The Cattle Trading Network and Its Effect on the Spread of Brucellosis in Paraná, Brazil. *Ruminants*, *3*(3), 202-213.

Rogan, W. J., & Gladen, B. (1978). Estimating prevalence from the results of a screening test. *American Journal of Epidemiology*, *107*(1), 71-76. https://doi.org/10.1093/oxfordjournals.aje.a112510

Roth, F., Zinsstag, J., Orkhon, D., Chimed-Ochir, G., Hutton, G., Cosivi, O., Carrin, G., & Otte, J. (2003). Human health benefits from livestock vaccination for brucellosis: case study. *Bulletin of the World Health Organization*, *81*(12), 867-876. https://stacks.cdc.gov/view/cdc/16299

Rushton, J., Thornton, P. K., & Otte, M. J. (1999). Methods of economic impact assessment. *Revue scientifique et technique-Office international des épizooties*, *18*, 315-342.

Santos, R. L., Martins, T. M., Borges, Á. M., & Paixão, T. A. (2013). Economic losses due to bovine brucellosis in Brazil. *Pesquisa Veterinária Brasileira*, *33*, 759-764. https://doi.org/10.1590/S0100-736X2013000600012

Sargeant, J. M., O'Connor, A. M., Dohoo, I. R., Erb, H. N., Cevallos, M., Egger, M., Ersbøll, A. K., Martin, S. W., Nielsen, L. R., Pearl, D. L., Pfeiffer, D. U., Sanchez, J., Torrence, M. E., Vigre, H., Waldner, C., & Ward, M. P. (2016). Methods and processes of developing the strengthening the reporting of observational studies in epidemiology − veterinary

(STROBE-Vet) statement. *Preventive Veterinary Medicine*, *134*, 188-196. https://doi.org/10.1016/j.prevetmed.2016.09.005

Sarrazin, S., Cay, A. B., Laureyns, J., & Dewulf, J. (2014). A survey on biosecurity and management practices in selected Belgian cattle farms. *Preventive Veterinary Medicine*, *117*(1), 129-139. https://doi.org/10.1016/j.prevetmed.2014.07.014

Savini, L., Candeloro, L., Conte, A., De Massis, F., & Giovannini, A. (2017). Development of a forecasting model for brucellosis spreading in the Italian cattle trade network aimed to prioritise the field interventions. *PLOS ONE*, *12*(6), e0177313. https://doi.org/10.1371/journal.pone.0177313

Sayers, R. G., Sayers, G. P., Mee, J. F., Good, M., Bermingham, M. L., Grant, J., & Dillon, P. G. (2013). Implementing biosecurity measures on dairy farms in Ireland. *The Veterinary Journal*, *197*(2), 259-267. https://doi.org/10.1016/j.tvjl.2012.11.017

Seleem, M. N., Boyle, S. M., & Sriranganathan, N. (2010). Brucellosis: A re-emerging zoonosis. *Veterinary Microbiology*, *140*(3), 392-398. https://doi.org/10.1016/j.vetmic.2009.06.021

Shaukat, W., de Jong, E., McCubbin, K. D., Biesheuvel, M. M., van der Meer, F. J. U. M., De Buck, J., Lhermie, G., Hall, D. C., Kalbfleisch, K. N., Kastelic, J. P., Orsel, K., & Barkema, H. W. (2024). Herd-level prevalence of bovine leukemia virus, Salmonella Dublin and Neospora caninum in Alberta, Canada, dairy herds using ELISA on bulk tank milk samples. *Journal of Dairy Science*, *107*(10), 8313-8328. https://doi.org/10.3168/jds.2023-24611

Shi, F., Huang, B., Shen, C., Liu, Y., Liu, X., Fan, Z., Mubarik, S., Yu, C., & Sun, X. (2021). Characterization and influencing factors of the pig movement network in Hunan Province, China. *Preventive Veterinary Medicine*, *193*, 105396. https://doi.org/10.1016/j.prevetmed.2021.105396

Shortall, O., Green, M., Brennan, M., Wapenaar, W., & Kaler, J. (2017). Exploring expert opinion on the practicality and effectiveness of biosecurity measures on dairy farms in the United Kingdom using choice modeling. *Journal of Dairy Science*, *100*(3), 2225-2239. https://doi.org/10.3168/jds.2016-11435

Shortall, O., Ruston, A., Green, M., Brennan, M., Wapenaar, W., & Kaler, J. (2016). Broken biosecurity? Veterinarians' framing of biosecurity on dairy farms in England. *Preventive Veterinary Medicine*, *132*, 20-31. https://doi.org/10.1016/j.prevetmed.2016.06.001

Sibhat, B., Tessema, T. S., Nile, E., & Asmare, K. (2022). Brucellosis in Ethiopia: A comprehensive review of literature from the year 2000–2020 and the way forward. *Transboundary and Emerging Diseases*, *69*(5), e1231-e1252. https://doi.org/10.1111/tbed.14495

Singh, B. B., Dhand, N. K., & Gill, J. P. S. (2015). Economic losses occurring due to brucellosis in Indian livestock populations. *Preventive Veterinary Medicine*, *119*(3), 211-215.

https://doi.org/10.1016/j.prevetmed.2015.03.013

Singh, B. B., Kostoulas, P., Gill, J. P. S., & Dhand, N. K. (2018). Cost-benefit analysis of intervention policies for prevention and control of brucellosis in India. *PLoS Negl Trop Dis*, *12*(5), e0006488. https://doi.org/10.1371/journal.pntd.0006488

Smith, R. L., Grohn, Y. T., Pradhan, A. K., Whitlock, R. H., Van Kessel, J. S., Smith, J. M., Wolfgang, D. R., & Schukken, Y. H. (2009). A longitudinal study on the impact of Johne's disease status on milk production in individual cows. *Journal of Dairy Science*, *92*(6), 2653-2661. https://doi.org/10.3168/jds.2008-1832

Sorin-Dupont, B., Picault, S., Pardon, B., Ezanno, P., & Assié, S. (2023). Modeling the effects of farming practices on bovine respiratory disease in a multi-batch cattle fattening farm. *Preventive Veterinary Medicine*, *219*, 106009. https://doi.org/10.1016/j.prevetmed.2023.106009

Stevenson, M. (2021). Sample Size Estimation in Veterinary Epidemiologic Research. *Frontiers in Veterinary Science*, *7*. https://www.frontiersin.org/articles/10.3389/fvets.2020.539573

Stevenson, M., Sergeant, E., Nunes, T., Heuer, C., Marshall, J., Sanchez, J., Thornton, R., Reiczigel, J., Robison-Cox, J., Sebastiani, P., Solymos, P., Yoshida, K., Jones, G., Pirikahu, S., Firestone, S., Kyle, R., Popp, J., Jay, M., Reynard, C., Cheung, A., Singanallur, N., Szabo, A., & Rabiee, A. (2022). *epiR: Tools for the Analysis of Epidemiological Data. R package version 2.0.65*. https://CRAN.R-project.org/package=epiR

Su, Y.-S., & Yajima, M. (2021). R2jags: Using R to run 'JAGS'. *R package version 0.7-1*. https://cran.r-project.org/web/packages/R2jags/index.html

Szyda, J., & Komisarek, J. (2007). Statistical Modeling of Candidate Gene Effects on Milk Production Traits in Dairy Cattle. *Journal of Dairy Science*, *90*(6), 2971-2979. https://doi.org/10.3168/jds.2006-724

Tang, H., Fournié, G., Li, J., Zou, L., Shen, C., Wang, Y., Cai, C., Edwards, J., Robertson, I. D., Huang, B., & Bruce, M. (2022a). Analysis of the movement of live broilers in Guangxi, China and implications for avian influenza control. *Transboundary and Emerging Diseases*, *69*(4), e775-e787. https://doi.org/10.1111/tbed.14351

Tang, H., Kang, J., Shen, C., Wang, Y., Robertson, I. D., Cai, C., Edwards, J., Huang, B., & Bruce, M. (2022b). Benefit-cost analysis of a H7N9 vaccination program in poultry in Guangxi, China. *Preventive Veterinary Medicine*, *200*, 105580. https://doi.org/10.1016/j.prevetmed.2022.105580

Tao, X., Qiu, L., Chen, N., Xie, M., & Wang, W. (2016). Epidemiological survey of animal brucellosis in Chuxiong Prefecture in 2015. *Shanghai Journal of Animal Husbandry and Veterinary Medicine*(03), 19.

The Center for Food Security and Public Health. (2018). *Brucellosis: Brucella abortus, Bovine Brucellosis, Undulant Fever, Contagious Abortion, Bang's Disease*. https://www.cfsph.iastate.edu/Factsheets/pdfs/brucellosis_abortus.pdf

The World Bank. (2022). *Official exchange rate (LCU per US$, period average)*. https://data.worldbank.org/indicator/PA.NUS.FCRF

The World Bank. (2023). *Inflation, consumer prices (annual %) - China*. International Monetary Fund. https://data.worldbank.org/indicator/FP.CPI.TOTL.ZG?locations=CN

Timonen, A. A. E., Katholm, J., Petersen, A., Mõtus, K., & Kalmus, P. (2017). Within-herd prevalence of intramammary infection caused by Mycoplasma bovis and associations between cow udder health, milk yield, and composition. *Journal of Dairy Science*, *100*(8), 6554-6561. https://doi.org/10.3168/jds.2016-12267

Toftaker, I., Ågren, E., Stokstad, M., Nødtvedt, A., & Frössling, J. (2020). Herd level estimation of probability of disease freedom applied on the Norwegian control program for bovine respiratory syncytial virus and bovine coronavirus. *Preventive Veterinary Medicine*, *181*, 104494. https://doi.org/https://doi.org/10.1016/j.prevetmed.2018.07.002

Tukana, A., & Gummow, B. (2017). Dairy farm demographics and management factors that played a role in the re-emergence of brucellosis on dairy cattle farms in Fiji. *Tropical Animal Health and Production*, *49*(6), 1171-1178. https://doi.org/10.1007/s11250-017-1314-8

Van Emon, M., Sanford, C., & McCoski, S. (2020). Impacts of Bovine Trace Mineral Supplementation on Maternal and Offspring Production and Health. *Animals*, *10*(12).

Wainaina, M., Aboge, G. O., Omwenga, I., Ngaywa, C., Ngwili, N., Kiara, H., Wamwere-Njoroge, G., & Bett, B. (2020). Detection of Brucella spp. in raw milk from various livestock species raised under pastoral production systems in Isiolo and Marsabit Counties, northern Kenya. *Tropical Animal Health and Production*, *52*(6), 3537-3544. https://doi.org/10.1007/s11250-020-02389-1

Wan, J. (2017). A investigation on prevalence of brucellosis in dairy cows in one area of Henan Province. *China Herbivore Science*, *37*(03), 75-76.

Wang, H., Xu, W., Zhu, K., Zhu, S., Zhang, H., Wang, J., Yang, Y., Shao, F., Jiang, N., Tao, Z., Jin, H., Tang, Y., Huo, L., Dong, F., Li, Z., Ding, H., & Liu, Z. (2020a). Molecular investigation of infection sources and transmission chains of brucellosis in Zhejiang, China. *Emerging Microbes & Infections*, *9*(1), 889-899. https://doi.org/10.1080/22221751.2020.1754137

Wang, X., & Jiang, H. (2020b). Global prevalence of human brucellosis. *Zhonghua Liu Xing Bing Xue Za Zhi*, *41*(10), 1717-1722. https://doi.org/10.3760/cma.j.cn112338-20191022-00751

Wang, X., Xu, K., Zhang, J., Feng, P., Gao, X., & Zhao, K. (2021a). Risk Factors Related to

Brucella Infection in Occupational Exposure Population at High Risk in Cattle/Sheep Farms in Xi'an City of Shaanxi Province. *China Animal Health Inspection*, *38*(05), 5-9.

Wang, Y., Robertson, I. D., Cheng, S., Wang, Y., Hou, L., Wang, G., Wu, X., Li, X., Chen, Y., & Guo, A. (2020c). 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. https://doi.org/10.1016/j.prevetmed.2020.105086

Wang, Y., Vallée, E., Compton, C., Heuer, C., Guo, A., Wang, Y., Zhang, Z., & Vignes, M. (2024a). A novel Bayesian Latent Class Model (BLCM) evaluates multiple continuous and binary tests: A case study for Brucella abortus in dairy cattle. *Preventive Veterinary Medicine*, *224*, 106115. https://doi.org/10.1016/j.prevetmed.2024.106115

Wang, Y., Vallée, E., Heuer, C., Wang, Y., Guo, A., Zhang, Z., & Compton, C. (2024b). A scoping review on the epidemiology and public significance of Brucella abortus in Chinese dairy cattle and humans. *One Health*, *18*, 100683. https://doi.org/10.1016/j.onehlt.2024.100683

Wang, Y., Wang, Y., Peng, Q., Xiang, Z., Chen, Y., Wang, G., Wu, X., Guo, A., & Robertson, I. D. (2022). A case study investigating the effects of emergency vaccination with Brucella abortus A19 vaccine on a dairy farm undergoing an abortion outbreak in China. *Animal Diseases*, *2*(1), 24. https://doi.org/10.1186/s44149-022-00056-6

Wang, Y., Wang, Y., Zhang, L., Wang, A., Yan, Y., Chen, Y., Li, X., Guo, A., & Robertson, I. D. (2021b). An epidemiological study of brucellosis on mainland China during 2004–2018. *Transboundary and Emerging Diseases*, *68*(4), 2353-2363. https://doi.org/10.1111/tbed.13896

Wareth, G., Melzer, F., El-Diasty, M., Schmoock, G., Elbauomy, E., Abdel-Hamid, N., Sayour, A., & Neubauer, H. (2017). Isolation of Brucella abortus from a Dog and a Cat Confirms their Biological Role in Re-emergence and Dissemination of Bovine Brucellosis on Dairy Farms. *Transboundary and Emerging Diseases*, *64*(5), e27-e30. https://doi.org/10.1111/tbed.12535

Whatmore, A. M., & Foster, J. T. (2021). Emerging diversity and ongoing expansion of the genus Brucella. *Infection, Genetics and Evolution*, *92*, 104865. https://doi.org/10.1016/j.meegid.2021.104865

Wickham, H. (2016). Getting Started with ggplot2. In H. Wickham (Ed.), *ggplot2: Elegant Graphics for Data Analysis* (pp. 11-31). Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4_2

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D. A., François, R., Grolemund, G., Hayes, A., Henry, L., & Hester, J. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686.

WOAH. (2022a). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2022 -*

Chapter 2.2.5. Statistical approaches to validation. Retrieved 10 August from https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/2.02.05_STATISTICAL_VALIDATION.pdf

WOAH. (2022b). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2022 - Chapter 3.1.4. – Brucellosis (infection with Brucella abortus, B. melitensis and B. suis)* Retrieved 10 Augest from https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf

WOAH. (2023a, May 2023). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals - Chapter 1.1.6. Validation of diagnostic assays for infectious diseases of terrestrial animals*. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.06_VALIDATION.pdf

WOAH. (2023b). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals - Chapter 3.1.4. Brucellosis (infection with Brucella abortus, B. melitensis and B. suis)*. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf

Wood, S. N. (2017). *Generalized Additive Models: An Introduction with R, Second Edition (2nd ed.)*. Chapman and Hall/CRC. https://doi.org/10.1201/9781315370279

Wu, J. J., Wathes, D. C., Brickell, J. S., Yang, L. G., Cheng, Z., Zhao, H. Q., Xu, Y. J., & Zhang, S. J. (2012). Reproductive performance and survival of Chinese Holstein dairy cows in central China. *Animal Production Science*, *52*(1), 11-19. https://doi.org/10.1071/AN11146

Wu, S. (2021). *Epidemiological Investigation and Analysis of Brucellosis in Ulanqab City of Inner Mongolia* [Master, Inner Mongolia Agricultural University].

Wu, X. (2022). *Epidemiological investigation and prevention measures of bovine brucellosis in Urumqi* [Master, Xinjiang Agricultural University].

Wu, Z., Cao, X., Lin, X., Zhang, J., Zhang, L., Jin, S., & Wang, L. (2018). Surveillance, Isolation and Identification of Brucella Surveillence in Cows in Yongjing County of Gansu Province. *China Animal Health Inspection*, *35*(10), 83-86+89.

Xavier, M. N., Paixão, T. A., Poester, F. P., Lage, A. P., & Santos, R. L. (2009). Pathological, Immunohistochemical and Bacteriological Study of Tissues and Milk of Cows and Fetuses Experimentally Infected with Brucella abortus. *Journal of Comparative Pathology*, *140*(2), 149-157. https://doi.org/10.1016/j.jcpa.2008.10.004

Xiao, Y., Zou, G., Yin, J., Tan, W., Zhou, J., & Zhang, H. (2016). Seroepidemiology of human Brucella infection in Yixing, China. *Tropical Doctor*, *47*(2), 165-167. https://doi.org/10.1177/0049475516640191

Xie, T., Xiong, H., Wang, D., Luo, C., Liu, G., Li, C., Ren, K., Hao, Y., & Li, S. (2022). Study on changes in body weight and sizes of Chinese Holstein dairy cows with parity, lactation and gestational age. *Chinese Journal of Animal Science*, *58*(04), 84-89.

Xue, H., Li, J., Ma, L., Yang, X., Ren, L., Zhao, Z., Wang, J., Zhao, Y., Zhao, Z., Zhang, X., Liu, Z., & Li, Z. (2023). Seroprevalence and Molecular Characterization of Brucella abortus from the Himalayan Marmot in Qinghai, China. *Infection and Drug Resistance*, *16*(null), 7721-7734. https://doi.org/10.2147/IDR.S436950

Yan, G., Pang, Z., Hu, Y., Zhou, Z., Liu, H., Luo, Y., Ren, Z., Ma, X., Cao, S., Shen, L., Wang, Y., Gou, L., Cai, D., Zhu, Y., Zhong, Y., Li, W., Shi, X., Peng, G., & Zhong, Z. (2022). First Isolation and Multilocus Sequence Typing of Brucella canis from a Subclinically Infected Pet Dog in China. *Veterinary Sciences*, *9*(1).

Yang, C., Gao, J., Xian, R., Liu, X., Kuai, W., Yin, C., Fan, H., Tian, J., Ma, X., & Ma, J. (2024). Molecular epidemiology of Brucella abortus isolated from the environment in Ningxia Hui autonomous region, China. *Infection, Genetics and Evolution*, *123*, 105635. https://doi.org/https://doi.org/10.1016/j.meegid.2024.105635

Yang, D. A., & Laven, R. A. (2022a). Performance of the StaphGold ELISA test in determining subclinical Staphylococcus aureus infections in dairy cows using a Gaussian mixture model. *Veterinary Medicine and Science*, *8*(4), 1632-1639. https://doi.org/10.1002/vms3.785

Yang, D. A., Xiao, X., Jiang, P., Pfeiffer, D. U., & Laven, R. A. (2022b). Keeping continuous diagnostic data continuous: Application of Bayesian latent class models in veterinary research. *Preventive Veterinary Medicine*, *201*, 105596. https://doi.org/10.1016/j.prevetmed.2022.105596

Yang, J., He, C., Zhang, H., Liu, M., Zhao, H., Ren, L., Wu, D., Du, F., Liu, B., Han, X., He, S., & Chen, Z. (2021). Evaluation and Differential Diagnosis of a Genetic Marked Brucella Vaccine A19ΔvirB12 for Cattle. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.679560

Yang, R., Lv, L., & Lv, Y. (2009). Surveillance Report on Brucellosis in 2007 in Quzhou Municipality. *Chinese Primary Health Care*, *23*(05), 64-65.

Yang, X., Wang, Y., Li, J., Chen, J., Liu, J., Tian, G., Zhao, H., Piao, D., Fan, Y., & Jiang, H. (2022c). Genetic characteristics of an amikacin-resistant Brucella abortus strain first isolated from Marmota himalayana. *Microbial Pathogenesis*, *164*, 105402. https://doi.org/https://doi.org/10.1016/j.micpath.2022.105402

Yao, M., Guo, X., Wu, X., Bai, Q., Sun, M., & Yin, D. (2022). Evaluation of the Combined Use of Major Outer Membrane Proteins in the Serodiagnosis of Brucellosis. *Infect Drug Resist*, *15*, 4093-4100. https://doi.org/10.2147/idr.S372411

Youden, W. J. (1950). Index for rating diagnostic tests. *Cancer*, *3*(1), 32-35.

Youssef, D. M., Wieland, B., Knight, G. M., Lines, J., & Naylor, N. R. (2021). The effectiveness of biosecurity interventions in reducing the transmission of bacteria from livestock to humans at the farm level: A systematic literature review. *Zoonoses and Public Health*, *68*(6), 549-562. https://doi.org/10.1111/zph.12807

Yu, X., Shentu, P., & Chen, L. (2011). Monitoring data analysis of Brucellosis in Jinhua from 2004 to 2010. *Chinese Rural Health Service Administration*, *31*(10), 1060-1061.

Zeng, H., Wang, Y., Sun, X., Liu, P., Xu, Q., Huang, D., Gao, L., You, S., & Huang, B. (2019a). Status and influencing factors of farmers' private investment in the prevention and control of sheep brucellosis in China: A cross-sectional study. *PLOS Neglected Tropical Diseases*, *13*(3), e0007285. https://doi.org/10.1371/journal.pntd.0007285

Zeng, J., Duoji, C., Yuan, Z., Yuzhen, S., Fan, W., Tian, L., Cai, C., & Robertson, I. (2017). Seroprevalence and risk factors for bovine brucellosis in domestic yaks (Bos grunniens) in Tibet, China. *Tropical Animal Health and Production*, *49*(7), 1339-1344. https://doi.org/10.1007/s11250-017-1331-7

Zeng, J., Robertson, I., Ji, Q., Dawa, Y., & Bruce, M. (2019b). Evaluation of the economic impact of brucellosis in domestic yaks of Tibet. *Transboundary and Emerging Diseases*, *66*(1), 476-487. https://doi.org/10.1111/tbed.13049

Zeng, J. Y., Ciren, D. J., Yundan, D. Z., Pu, Q., Gongjue, C. W., Jiumei, D. J., & Robertson, I. D. (2018). A study of the knowledge, attitudes and practices of Tibetan yak herders with respect to brucellosis. *International health*, *10*(4), 294-301.

Zhang, H., Deng, X., Cui, B., Shao, Z., Zhao, X., Yang, Q., Song, S., Wang, Z., Wang, Y., Wang, Y., Liu, Z., Sheng, J., & Chen, C. (2020). Abortion and various associated risk factors in dairy cow and sheep in Ili, China. *PLOS ONE*, *15*(10), e0232568-e0232568. https://doi.org/10.1371/journal.pone.0232568

Zhang, J., Xu, J., Fang, L., Yu, S., & Sun, G. (2016). Investigation and analysis of epidemic situation of brucellosis in livestock in Heilongjiang province. *Heilongjiang Animal Science and Veterinary Medicine*(10), 104-106.

Zhang, N., Huang, D., Wu, W., Liu, J., Liang, F., Zhou, B., & Guan, P. (2018). Animal brucellosis control or eradication programs worldwide: A systematic review of experiences and lessons learned. *Preventive Veterinary Medicine*, *160*, 105-115. https://doi.org/10.1016/j.prevetmed.2018.10.002

Zhang, N., Zhou, H., Huang, D. S., & Guan, P. (2019). Brucellosis awareness and knowledge in communities worldwide: A systematic review and meta-analysis of 79 observational studies. *PLOS Neglected Tropical Diseases*, *13*(5), e0007366. https://doi.org/10.1371/journal.pntd.0007366

Zhao, C., Liu, K., Jiang, C., Wei, X., Song, S., Wu, X., Wen, X., Fu, T., Shen, L., Shao, Z., & Li, Q. (2022). Epidemic characteristics and transmission risk prediction of brucellosis in Xi'an city, Northwest China. *Frontiers in Public Health*, *10*.

https://doi.org/10.3389/fpubh.2022.926812

Zhao, S., Wang, S., Zhao, P., Yuan, S., Fang, D., Guo, L., & Ban, F. (2020). Investigation on brucellosis infection of dairy cows in Henan Province in 2018. *Chinese Journal of Veterinary Medicine*, *56*(05), 42-44.

Zheludkov, M. M., & Tsirelson, L. E. (2010). Reservoirs of Brucella infection in nature. *Biology Bulletin*, *37*(7), 709-715. https://doi.org/10.1134/S106235901007006X

Zhou, X., Jiang, G., Liu, H., Hu, Y., Ren, S., & Li, X. (2014). Seropepidemiological survey of brucellosis among dairy farmers in Xindu District from 2010 to 2012. *Journal of Preventive Medicine Information*, *30*(07), 580-582.

Zhu, X., Wang, J., Zhang, Z., Yan, L., Liu, H., Chen, Y., Robertson, I. D., Guo, A., & Aleri, J. (2023a). A participatory approach to understand the attitudes and perceptions towards priority endemic cattle diseases among dairy farmers and animal health experts in Henan province, China. *Preventive Veterinary Medicine*, *218*, 105994. https://doi.org/10.1016/j.prevetmed.2023.105994

Zhu, X., Wang, J., Zhao, Y., Zhang, Z., Yan, L., Xue, Y., Chen, Y., Robertson, I. D., Guo, A., & Aleri, J. (2023b). Prevalence, distribution, and risk factors of bovine tuberculosis in dairy cattle in central China. *Preventive Veterinary Medicine*, *213*, 105887. https://doi.org/10.1016/j.prevetmed.2023.105887

Zhu, X., Wang, M., Zheng, X., & Zhang, P. (2019). Epidemiological Investigation of Livestock Brucellosis in Gaoling District. *Journal of Animal Science and Veterinary Medicine*, *38*(05), 42-44.

Zhu, X., Zhao, Z., Ma, S., Guo, Z., Wang, M., Li, Z., & Liu, Z. (2020). *Brucella melitensis*, a latent “travel bacterium,” continual spread and expansion from Northern to Southern China and its relationship to worldwide lineages. *Emerging Microbes & Infections*, *9*(1), 1618-1627. https://doi.org/10.1080/22221751.2020.1788995

Zhuo, M., Zhang, Z., Huang, J., Zhu, H., Wang, L., Lin, D., & Chen, L. (2007). Investigation report on human brucellosis in Nanping, Fujian province. *Disease Surveillance*(07), 498-499.

Zinsstag, J., Schelling, E., Roth, F., Bonfoh, B., De Savigny, D., & Tanner, M. (2007). Human benefits of animal interventions for zoonosis control. *Emerging infectious diseases*, *13*(4), 527.

Zinsstag, J., Schelling, E., Waltner-Toews, D., & Tanner, M. (2011). From “one medicine” to “one health” and systemic approaches to health and well-being. *Preventive Veterinary Medicine*, *101*(3), 148-156. https://doi.org/10.1016/j.prevetmed.2010.07.003

Appendix 1

Table S2.1 Specific literature searching strategy for four Databases (PubMed, Web of Science, Scopus, China National Knowledge Infrastructure - CNKI).

Database: PubMed	**Database-tailored syntax (using Boolean operators)**
Term	
1 disease	((((brucellosis[Title/Abstract]) OR (*Brucella* spp. [Title/Abstract])) OR (Bang's disease[Title/Abstract])) OR (undulant fever[Title/Abstract])) OR (Malta fever[Title/Abstract])
	AND
2 outcome	((((((((prevalence[Title/Abstract]) OR (seroprevalence[Title/Abstract])) OR (incidence[Title/Abstract])) OR (epidemiolog*[Title/Abstract])) OR (risk factors[Title/Abstract])) OR (surveillance[Title/Abstract])) OR (isolat*[Title/Abstract])) OR (molecular[Title/Abstract])) OR (identification[Title/Abstract])
	AND
3 population	(((((((((dairy[Title/Abstract]) OR (cow?[Title/Abstract])) OR (cattle[Title/Abstract])) OR (bovine[Title/Abstract])) OR (calf OR calves[Title/Abstract])) OR (heifer?[Title/Abstract])) OR (human?[Title/Abstract])) OR (people[Title/Abstract])) OR (patient?[Title/Abstract])) OR (person?[Title/Abstract])
	AND
4 country	(China[Title/Abstract]) OR (Chinese[Title/Abstract])
	AND
5 time period	("2004/01/01"[Date - Publication] : "2022/12/31"[Date - Publication])
Total number of identified in PubMed	155

Database: Web of Science (WOS)	**Database-tailored syntax (using Boolean operators)**

Term	
1 disease	TS = (brucellosis OR Brucella OR Bang's disease OR undulant fever OR Malta fever)
	AND
2 outcome	TS=(prevalence OR seroprevalence OR incidence OR epidemiolog* OR risk factor? OR surveillance OR isolat* OR molecular OR identification)
	AND
3 population	TS=(dairy OR cow? OR cattle OR bovine OR calf OR calves OR heifer? OR human? OR people OR patient? OR person?)
	AND
4 country	TS=(China OR Chinese)
	AND
5 time period	DOP=2004-01-01 to 2022-12-31
Total number of identified	143

Database: Scopus	**Database-tailored syntax (using Boolean operators)**
Term	
1 disease	brucellosis OR brucella OR bang's disease OR undulant AND fever OR malta AND fever
	AND
2 areas	prevalence OR seroprevalence OR incidence OR epidemiolog* OR risk factor OR surveillance OR isolat* OR molecular OR identification
	AND
3 population	dairy OR cattle OR cow OR bovine OR human OR people OR patient OR person
	AND
4 country	China OR Chinese
	AND
5 time period	PUBYEAR > 2003 AND PUBYEAR < 2023

	AND
6 publication type	LIMIT-TO (DOCTYPE , "ar") OR LIMIT-TO (DOCTYPE , "sh")
Total number of identified in Scopus	250

Database: CNKI	**Advanced search (using Boolean operators)**
Term	
1 disease	布鲁氏菌 + 流产布鲁氏杆菌 + 布鲁氏菌病 + 布氏菌属 + 布氏菌 + 布病
	AND
2 areas	流行病学 + 流行率 + 发病率 + 风险因子 + 分离 + 鉴定 + 分型
	AND
3 species	奶牛场 + 牛 + 奶牛 + 犊牛 + 青年牛 + 母牛 + 人
	AND
4 country	中国
	AND
5 time period	2004-01-01 to 2022-12-31
Total number of identified in CNKI	824

Appendix 2

Table S 3.1 Eight scenarios used for sensitivity analysis include changes in prior distribution of sensitivity and specificity of RBT and SAT, and region-specific prevalence, while other parameters remain constant across all scenarios as described in Table 3.1.

Scenarios	Prevalence in PR	Prevalence in ZR	SeRBT	SpRBT	SeSATc[i] (i = 1, 2)	SpSATc[i] (i = 1, 2)
Scenario 1	B (1.14, 14.56)	B (1.06, 1.32)	B (13.58, 3.91) T(1-SpRBT,)	B (9.92, 2.42)	B (21.40, 7.48) T(1-SpSATc[i],)	B (9.92, 2.42)
Scenario 2	B (1, 1)	B (1, 1)	B (13.58, 3.91) T(1-SpRBT,)	B (9.92, 2.42)	B (21.40, 7.48) T(1-SpSATc[i],)	B (9.92, 2.42)
Scenario 3	B (1.14, 14.56)	B (1.06, 1.32)	B (1, 1) T(1-SpRBT,)	B (1, 1)	B (21.40, 7.48) T(1-SpSATc[i],)	B (9.92, 2.42)
Scenario 4	B (1.14, 14.56)	B (1.06, 1.32)	B (13.58, 3.91) T(1-SpRBT,)	B (9.92, 2.42)	B (1, 1) T(1-SpSATc[i],)	B (1, 1)
Scenario 5	B (1, 1)	B (1, 1)	B (1, 1) T(1-SpRBT,)	B (1, 1)	B (21.40, 7.48) T(1-SpSATc[i],)	B (9.92, 2.42)
Scenario 6	B (1, 1)	B (1, 1)	B (13.58, 3.91) T(1-SpRBT,)	B (9.92, 2.42)	B (1, 1) T(1-SpSATc[i],)	B (1, 1)
Scenario 7	B (1.14, 14.56)	B (1.06, 1.32)	B (1, 1) T(1-SpRBT,)	B (1, 1)	B (1, 1) T(1-SpSATc[i],)	B (1, 1)
Scenario 8	B (1, 1)	B (1, 1)	B (1, 1) T(1-SpRBT,)	B (1, 1)	B (1, 1) T(1-SpSATc[i],)	B (1, 1)

Note: B means *Beta* distribution; T means truncation function; c[1] means RBT-negative conditions; c[2] means RBT-positive conditions.

Table S 3.2 The cross-classified animal-level counts of the dichotomous outcome of four tests for detection of antibodies against *Brucella* spp. in dairy cows. ‘POS’ and ‘NEG’ refer to the test positive and test negative status for each test. FPA and C-ELISA results are interpreted by kit-recommended cut-off values.

Test				Population	
RBT	SAT	FPA	C-ELISA	PR	ZR
NEG	NEG	NEG	NEG	312	236
POS	NEG	NEG	NEG	0	2
NEG	POS	NEG	NEG	0	1
POS	POS	NEG	NEG	0	0
NEG	NEG	POS	NEG	1	4
POS	NEG	POS	NEG	0	7
NEG	POS	POS	NEG	0	0
POS	POS	POS	NEG	0	0
NEG	NEG	NEG	POS	0	1
POS	NEG	NEG	POS	0	0
NEG	POS	NEG	POS	0	0
POS	POS	NEG	POS	0	0
NEG	NEG	POS	POS	1	3
POS	NEG	POS	POS	3	9
NEG	POS	POS	POS	0	0
POS	POS	POS	POS	4	67
Total				321	330

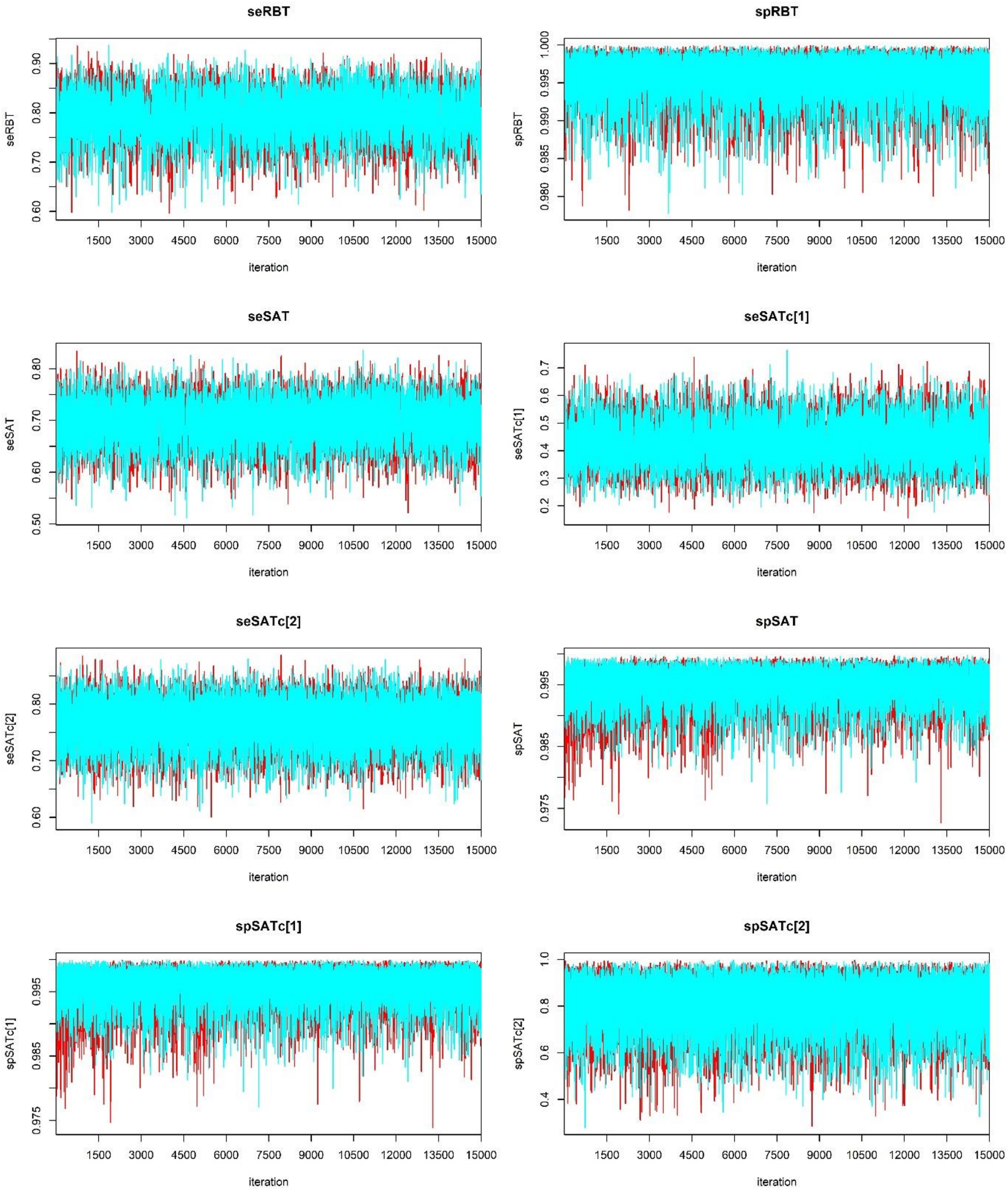


Figure S 3.1 The trace plot of sensitivity and specificity of RBT and SAT shows model convergence.

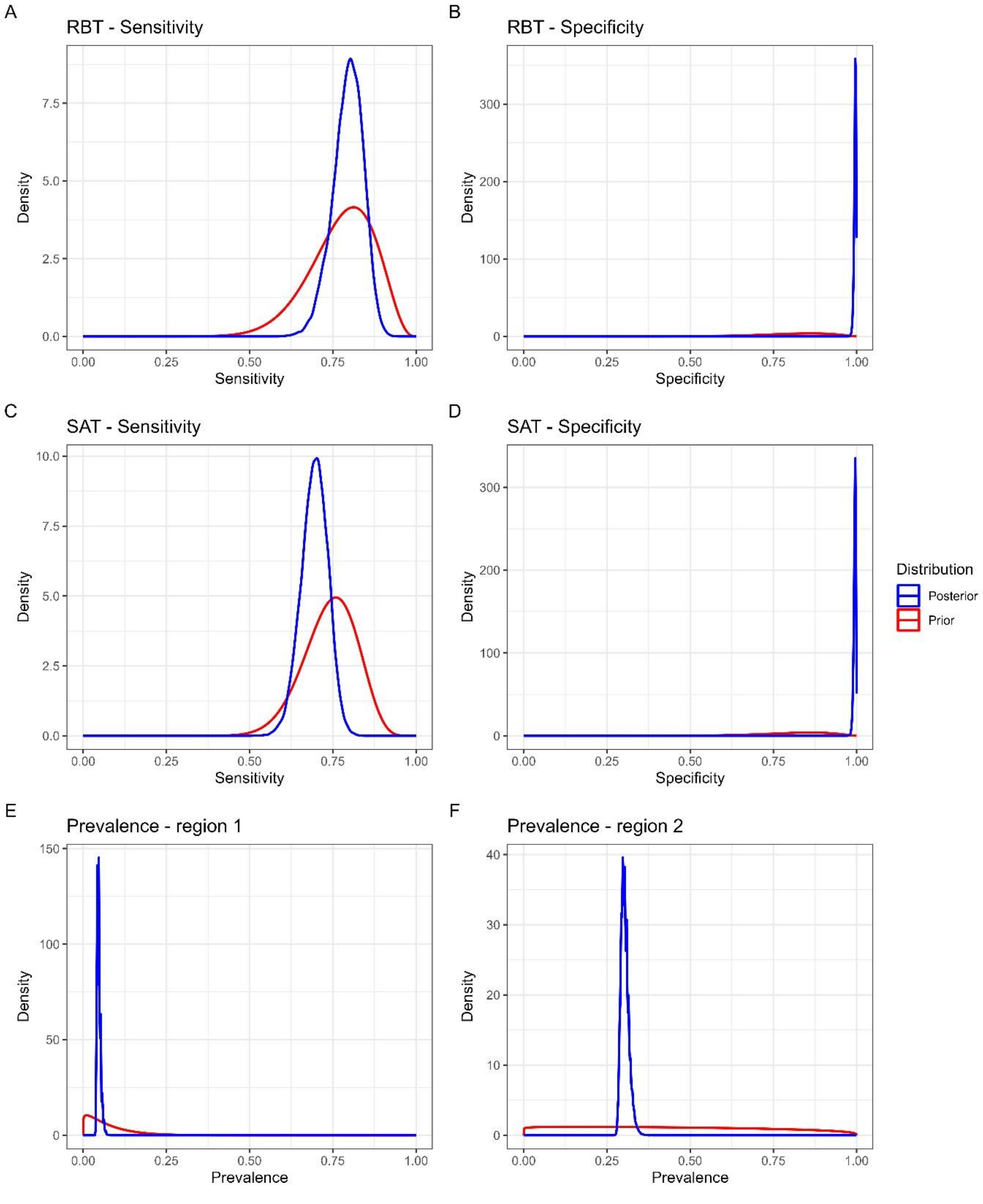


Figure S 3.2 A figure shows the prior and posterior distributions of sensitivity and specificity of RBT and SAT, region-specific prevalence.

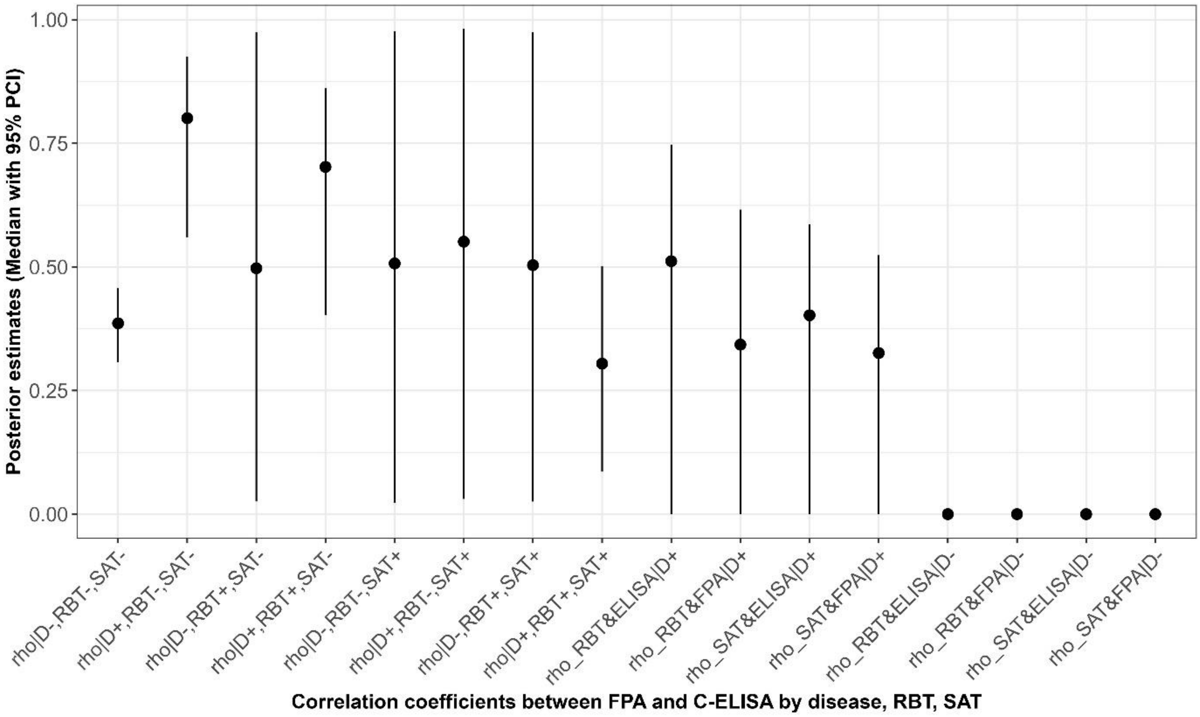


Figure S 3.3 A dot plot with bar (median, 95% posterior credible interval - PCI) about correlation coefficients among RBT, SAT, FPA and C-ELISA results depending on disease status (D). rho$_{[i]}$ (i = 1,2…8) represents coefficients between FPA and C-ELISA given conditions as follows: $_{[1]}$: D-, RBT-, SAT-; $_{[2]}$: D+, RBT-, SAT-; $_{[3]}$: D-, RBT+, SAT-; $_{[4]}$: D+, RBT+, SAT-; $_{[5]}$: D-, RBT-, SAT+; $_{[6]}$: D+, RBT-, SAT+; $_{[7]}$: D-, RBT+, SAT+; $_{[8]}$: D+, RBT+, SAT+.

Table S 3.3 Posterior distributions of the parameters of the Gaussian mixture model, presented with posterior median, 95% posterior credible intervals (PCI), and Rhat.

Parameters	Mean	Standard deviation	Median	95% PCI	Rhat
$alpha_1$	-4.71	0.55	-4.69	-5.83, -3.65	1.001
$alpha_2$	-2.24	0.47	-2.26	-3.16, -1.28	1.008
U_1	-0.69	0.59	-0.63	-2.01, 2.91	1.002
U_2	-0.45	0.55	-0.40	-1.66, 1.85	1.001
U_3	0.72	0.53	0.68	-0.19, 1.85	1.006
U_4	-0.20	0.43	-0.17	-1.11, -0.62	1.007
U_5	-0.39	0.44	-0.36	-1.32, 0.43	1.006
U_6	0.14	0.43	0.14	-0.75, 0.99	1.006
$\mu.mP_{[1]}$	-0.17	0.002	-0.17	-0.18, -0.17	1.001
$\mu.mP_{[2]}$	0.05	0.04	0.04	-0.03, 0.15	1.003
$\mu.mP_{[3]}$	-0.14	0.09	-0.14	-0.29, -0.006	1.001
$\mu.mP_{[4]}$	0.19	0.05	0.19	0.01, 0.28	1.001
$\mu.mP_{[5]}$	-0.16	0.08	-0.16	-0.20, -0.01	1.004
$\mu.mP_{[6]}$	0.17	0.23	0.15	-0.20, 0.57	1.113
$\mu.mP_{[7]}$	-0.14	0.09	-0.14	-0.29, -0.006	1.001
$\mu.mP_{[8]}$	0.39	0.02	0.39	0.34, 0.44	1.000
$\tau.mP_{[1]}$	447.09	37.63	443.30	10.59, 531.74	1.002
$\tau.mP_{[2]}$	24.81	8.68	23.92	10.59, 44.26	1.002
$\tau.mP_{[3]}$	0.002	0.009	0.0002	0.00007, 0.03	1.074
$\tau.mP_{[4]}$	24.96	7.79	24.05	12.16, 42.52	1.001
$\tau.mP_{[5]}$	17.47	3.84	17.83	6.41, 23.42	1.007
$\tau.mP_{[6]}$	9.96	3.00	7.55	2.16, 14.63	1.003
$\tau.mP_{[7]}$	0.003	0.001	0.0004	0.00003, 0.002	1.072

$\tau.mP_{[8]}$	25.24	4.26	24.97	17.56, 34.27	1.001
$\mu.PI_{[1]}$	-1.58	0.02	-1.58	-1.61, -1.55	1.000
$\mu.PI_{[2]}$	-1.43	0.14	-1.47	-1.58, -1.05	1.003
$\mu.PI_{[3]}$	-1.39	0.46	-1.33	-2.24, -0.73	1.001
$\mu.PI_{[4]}$	-0.62	0.12	-0.63	-0.85, -0.39	1.001
$\mu.PI_{[5]}$	-1.66	0.40	-1.73	-2.26, -0.79	1.026
$\mu.PI_{[6]}$	-0.85	0.63	-0.85	-1.90, 0.23	1.133
$\mu.PI_{[7]}$	-1.37	0.46	-1.31	-2.24, -0.72	1.001
$\mu.PI_{[8]}$	-0.06	0.02	-0.06	-0.10, -0.01	1.001
$\tau.PI_{[1]}$	8.59	0.60	8.58	7.47, 9.83	1.001
$\tau.PI_{[2]}$	0.68	0.23	0.65	0.29, 1.21	1.003
$\tau.PI_{[3]}$	0.007	0.003	0.006	0.002, 0.014	1.004
$\tau.PI_{[4]}$	3.61	1.13	3.48	1.76, 6.14	1.002
$\tau.PI_{[5]}$	8.07	2.11	7.82	4.56, 12.76	1.003
$\tau.PI_{[6]}$	7.26	3.31	7.80	1.69, 14.58	1.004
$\tau.PI_{[7]}$	0.012	0.006	0.023	0.001, 0.038	1.027
$\tau.PI_{[8]}$	27.55	4.70	27.27	19.08, 37.47	1.001

$alpha_i$ for i =1, 2, mean of the logit-transformation region-specific prevalence.

U_k for k = 1, 2, 3, 4, 5, 6, mean of the logit-normal farm-level random effect.

$\mu.mP_{[k]}$ for k = 1, 2,…8, mean of the transformed FPA results given conditions as follows: $_{[1]}$: D-, RBT-, SAT-; $_{[2]}$: D+, RBT-, SAT-; $_{[3]}$: D-, RBT+, SAT-; $_{[4]}$: D+, RBT+, SAT-; $_{[5]}$: D-, RBT-, SAT+; $_{[6]}$: D+, RBT-, SAT+; $_{[7]}$: D-, RBT+, SAT+; $_{[8]}$: D+, RBT+, SAT+.

$\tau.mP_{[k]}$ for k = 1, 2,…8, precision of the transformed FPA results given conditions as above.

$\mu.PI_{[k]}$ for k = 1, 2,…8, mean of the transformed C-ELISA results given conditions as above.

$\tau.PI_{[k]}$ for k = 1, 2,…8, precision of the transformed C-ELISA results given conditions as above.

Table S 3.4 Table about parity frequency of the sampled population within each dairy herd and region

	Number (proportion within each herd or region, %)					
	Parity 0	Parity 1	Parity 2	Parity 3	Parity 4	Overall
Farm 1	29 (27.4)	27 (25.5)	29 (27.3)	17 (16.0)	4 (3.8)	106
Farm 2	30 (26.8)	29 (25.9)	27 (24.1)	20 (17.9)	6 (5.3)	112
Farm 3	26 (25.2)	26 (25.2)	25 (24.3)	18 (17.5)	8 (7.8)	103
Farm 4	31 (27.4)	30 (26.5)	25 (22.1)	20 (17.7)	7 (6.2)	113
Farm 5	29 (26.4)	32 (29.1)	20 (18.2)	20 (18.2)	9 (8.2)	110
Farm 6	29 (27.1)	29 (27.1)	22 (20.6)	19 (17.7)	8 (7.5)	107
PR	85 (26.5)	82 (25.5)	81 (25.2)	55 (17.1)	18 (5.6)	321
ZR	89 (27.0)	91 (27.6)	67 (20.3)	59 (17.9)	24 (7.3)	330
Overall	174 (26.7)	173 (26.6)	148 (22.7)	114 (17.5)	42 (6.5)	651

Appendix 3

Questionnaire

All data obtained in this questionnaire will remain confidential and non-identifying. Please carefully read the **Information Sheet** and sign the attached **Consent Form** before completing this questionnaire if you agree to participate in this survey. This questionnaire will take about 20-30 minutes to complete. You are free to withdraw from this survey at any time and refuse to answer any questions.

Category 1: Demographic information	
Farm address	City___________District___________ Street/village_____________
Gender	☐ Male ☐ Female
Age	☐ Under 25 ☐ 25 to 34 ☐ 35 to 44 ☐ 45 to 54 ☐ 55 to 64 ☐ Over 65
Number of years farming dairy cattle	_______________
Education achievement	☐ No education ☐ Primary school ☐ High school ☐ Undergraduate or postgraduate
Work role within the farm	☐ Owner ☐ Manager ☐ Veterinarian ☐ Others, please specify_____
What was the average total number of animals on the farm during 2021-2022?	_______________(animals)
The average number of dairy cattle within each specific age group during 2021- 2022	☐ Calves (0-6 months) ______ ☐ Young heifers (6-12 months) ______ ☐ Replacement heifers (13-24 months) __ ☐ Mixed-age adult cows (>24 months) __
Category 2: Management and husbandry measures	
Please answer the following questions related to the **2021/2022 season (1 June 2021 to 31 May 2022)**	
What kind of mating methods did you use on your farm?	☐ Artificial insemination ☐ Natural mating ☐ Artificial insemination plus natural mating
Where are the inseminators coming from?	☐ Within dairy farm ☐ From outside the dairy farm
Which methods do you choose to do with culled cattle? (tick all applicable options)	☐ Send to the local slaughterhouse ☐ Sell to animal traders/markets

	☐ Sell to other dairy farms ☐ Others, please specify __________
Do you disinfect the calving places at least once per week?	☐ Yes ☐ No
Please specify whether raw milk feeding for calves is treated as follow?	☐ Direct feeding without processing ☐ Pasteurization ☐ Boiling
What kind of calving method does your farm use?	☐ Year-round calving ☐ Seasonal calving
How many total calves were born during the last year 2021-2022?	__________________
How many total calves were weaned during the last year 2021-2022?	__________________
How many total females were mated during the last year 2021-2022?	☐ Cows (>24 months)________________ ☐ Heifers (12-24 months)______________
Were any of the cows aborted or stillborn (dead at or within 24h of calving)?	☐ Yes ☐ No
If Yes, how many cows	__________________
If abortion occurred, what did you do with the aborted products?	☐ Left where it was ☐ Burn or bury it ☐ Fed it to other animals (i.e., dog)
How are aborted cattle typically treated?	☐ Manage like normally calving animals ☐ Conduct laboratory diagnosis and then make decision ☐ Without laboratory diagnosis, but isolation plus treatment as sick animals ☐ Sale ☐ Culling ☐ Others, please specify ______________
Where is the semen purchased from?	☐ Commercial company ☐ Bull semen from other farms ☐ Import semen ☐ Unknown
Category 3: Biosecurity practices with purchase and sale of animals, and contacts with visitors and vehicles, and wildlife.	
Purchased Cattle, please answer the following questions related to the **2021/2022 season (1 June 2021 to 31 May 2022)**	
Did your farm purchase any cattle during this period?	☐ Yes ☐ No
If Yes, what type of cattle do you usually purchase?	☐ Calves ☐ heifers ☐ Replacements ☐ adults
Did you test brucellosis for purchased animals?	☐ Yes

	☐ No
Where did you purchase cattle from?	☐ Neighbour farms ☐ Animal market ☐ Cattle traders ☐ Out-of-province suppliers
Did you isolate purchased cattle before mixing them into the herd?	☐ Yes, please specify the length days ☐ No
How often did you ask about the *Brucella* spp. disease status of the source herd(s)?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
How often did you ask about the *Brucella* spp. vaccination status of the source herds(s)?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
What factors influenced your decisions around adoption of *Brucella* spp. biosecurity for purchased cattle?	☐ Cost ☐ Lack of isolation shed(s) ☐ Increased workload ☐ Uncertainty about the role of isolation ☐ Other, specify______________
Sold Cattle, please answer the following questions related to **<u>2021/2022 season (1 June 2021 to 31 May 2022)</u>**	
Did you sell any cattle to other farms during this period	☐ Yes ☐ No
How often did the buyer(s) ask about the *Brucella* spp. disease status of your herd?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
How often did the buyer(s) ask about the *Brucella* spp. vaccination status of your herd?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
Where were the buyer(s) from?	☐ Neighbour farms ☐ Animal market ☐ Cattle trader(s) ☐ Restaurant(s) ☐ Abattoir
Contact with neighbour herds	
How far is the nearest dairy farm from your farm site?	______________Km
Are there cattle from other farms that are raised on your farm?	☐ Yes, please specify the number ______ ☐ No
How are these outside-farm animals raised?	☐ Separate shed, no direct contact ☐ Mixed/in-contact with your animals
What is the *Brucella* spp. status of the	☐ All negative ☐ All positive

neighbouring farms?	☐ Vaccinated ☐ Mixed positive and negative ☐ Don’t know
Do you share equipment, such as trailers or milk tankers, with your neighbour(s)?	☐ Yes ☐ No
Contact with visitors and vehicles	
Are there records of visitors and vehicles entering and exiting?	☐ Yes ☐ No
How often do you see uninvited visitors/vehicles passing through your farm?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
How often do people (e.g. vets, workers) who work with animals wash their boots and other equipment before and after contact with your cattle?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
Is there a disinfection channel at the entrance of the farm?	☐ Yes ☐ No
If Yes, how often are visitors disinfected?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
Is there a vehicle disinfection pool at the entrance of the farm?	☐ Yes ☐ No
If Yes, how often is the disinfectant in the vehicle disinfection pool being replaced?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
Contact with companion animals and wildlife	
Please select the following animals that exist on your farm (multiple-choice if applicable)	☐ None ☐ Beef ☐ Sheep ☐ Goat ☐ Cat ☐ Dog ☐ Chicken ☐ Other, specify__________
If Yes, how often did you see the above-ticked animals entering the production areas?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
How often did you see rodents (e.g. yellow weasel and mice) on your farm?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
How often were rat control programs conducted on your farm?	☐ Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always
Category 4: *Brucella* spp. testing and vaccination	
Please answer the following questions related to the **2021/2022 season (1 June 2021 to 31 May 2022)**	
Have any animals been tested for *Brucella* spp. in the last year?	☐ Yes ☐ No ☐ Unknown
If No What was the main reason for not performing testing? (select **ONE**)?	☐ Unaware of *Brucella* spp. ☐ Too expensive to test

		☐ Low perceived impact of *Brucella* spp. ☐ No intension to control ☐ Don’t know where to test ☐ Other:____________________________
If Yes	How often do you test for *Brucella* spp.?	☐ Annually ☐ Half a year ☐ Seasonally ☐ Occasionally ☐ Other:______________
	Which groups were usually tested?	☐ Aborted cows ☐ Replacement heifers ☐ Screening all animals ☐ Other:____________________________
	Did you have at least one positive test result in 2021-2022?	☐ Yes ☐ No ☐ Unknown
If test positive	Did you perform follow-up testing for undetected animals?	☐ Yes ☐ No ☐ Unknown
	How did you deal with animals with a positive test result?	☐ Culled ☐ Sold ☐ Remained in herd ☐ Other:__________ ☐ Unknown
Have you used any vaccines against *Brucella* spp. in past three years?		☐ Yes ☐ No ☐ Unknown
If Not, what was your primary reason why you did not use vaccine?		☐ Unaware of the efficacy of the vaccine ☐ Too expensive to vaccinate ☐ Policy limitation/regulation ☐ Concerns about vaccine interference with serological tests ☐ Other control measures
If Yes, when was the last time you vaccinated your animals?		_____Month______Year
Which vaccines did you choose to vaccinate your cattle?		☐ *Brucella abortus* A19 strain ☐ *Brucella abortus* A19-ΔVirB12 strain ☐ *Brucella suis* 2 strain ☐ Unknown
How much did it cost on average to vaccinate an animal?		________RMB______(vaccine+labor+cold storage)
Which of the following vaccination procedures did you use?		☐ Once at 6 months of age ☐ Once at 6 months of age and a booster before the first mating ☐ Once at 6 months of age and an annual booster before mating ☐ Other, please specify: ______________
Category 5: Motivations and challenges towards Henan *Brucella* spp. control		
Does anyone on the farm have symptoms like undulate fever or chronic arthritis (flu-like) in		☐ Yes ☐ No ☐ Unknown

2021-2022?	
Are you aware of the current Henan *Brucella* spp. control programs?	☐ Yes ☐ No
What do you think is the likelihood of achieving *Brucella* spp. control/eradication on your farm?	☐ Extremely unlikely ☐ Unlikely ☐ Neutral ☐ Likely ☐ Extremely likely
How likely are you to support the current *Brucella* spp. control projects in Henan Province?	☐ Extremely unlikely ☐ Unlikely ☐ Neutral ☐ Likely ☐ Extremely likely
Comments:	
Who do you think who should be the decision maker of Henan *Brucella* spp. control/eradication programs?	☐ Ministry of Agriculture and Rural Affairs ☐ Individual farmers ☐ Industry (Henan Dairy Association) ☐ Other:___________________
Comments:	
Please choose which *Brucella* spp. control measures should **<u>be voluntary</u>**? **(Multiple choice)** ☐ Purchasing *Brucella* spp. free or vaccinated animals only ☐ Isolation of animals brought into your farm ☐ Farm gate foot baths and other sanitation measures ☐ Testing moved animals off-site or/and on-site farm ☐ Testing all replacement calves after weaning ☐ Testing all replacement heifers before mating ☐ Vaccinating at-risk stock against *Brucella* spp. ☐ Vaccinating all animals	
Please choose which *Brucella* spp. control measures should **<u>be mandatory</u>**? **(Multiple choice)** ☐ Screening annually to establish *Brucella* spp. status of each herd ☐ Requiring herds to declare *Brucella* spp. status at the time of sale of cattle ☐ Restricting animal movements for test-positive farms ☐ Establishing a provincial database to record *Brucella* spp. status of each herd over time ☐ Adopting appropriate *Brucella* spp. control measures (e.g. test-and-culling with compensation) on test-positive farms ☐ Vaccinating all cattle at six months of age	
If adopt these control measures, what do you expect as the most significant benefits to eradicating *Brucella* spp. from your farm? (Multiple choice)	
☐ Reduce the risk of human infection ☐ Improve herd productivity ☐ Save disease prevention costs ☐ Increase the quality and price of milk	

☐ Others, please specify __________________
If adopt these control measures, what do you see as the most significant challenges to eradicating *Brucella* spp. from your farm? (Multiple choice)
☐ Huge cost input ☐ Require a long time ☐ Purchase replacement heifers to maintain stable herd structure ☐ Technical feasibility and practicability ☐ Others, please specify __________________
What features could be built into an eradication program to increase success? (Multiple choice)
☐ Establish a clear classification between vaccinated and non-vaccinated areas at the county or herd level ☐ Increase compensation for compulsory culling of animals ☐ Strengthen brucellosis testing before animal movement ☐ Increase government funding for routine disease surveillance ☐ Others, please specify __________________
Additional Comments

Thank you for your time and participation.

Appendix 4

Table S 4.1 Basic descriptions about samples in twelve selected Henan dairy herds

No. herd	Herd size	Operation type#	Breed	Calving pattern	No. animals re-sampled for this study	1st round of sampling period	2nd round of sampling period
1	700	Open	Holstein-Friesian	Year-round	107	21st Aug 2021 –18th Sep 2021	24th July 2022 – 19th Sep 2022
2	850	Open	Holstein-Friesian	Year-round	100	17th Aug 2021 – 22nd Sep 2021	17th Sep 2022 – 15th Oct 2022
3	220	Open	Holstein-Friesian	Year-round	25	30th Sep 2021 – 15th Oct 2021	24th Aug 2022 – 24th Oct 2022
4	3000	Closed	Holstein-Friesian	Seasonal	113	9th Nov 2021 – 16th Nov 2021	26th Oct 2022 – 3rd Dec 2022
5	1200	Closed	Holstein-Friesian	Seasonal	99	5th Aug 2021 – 21st Aug 2021	20th July 2022 – 12th Sep 2022
6	950	Open	Holstein-Friesian	Year-round	102	24th Aug 2021 – 30th Sep 2021	2nd Sep 2022 – 15th Oct 2022
7	1600	Open	Holstein-Friesian	Year-round	100	24th Aug 2021 – 7th Sep 2021	20th Aug 2022 – 17th Sep 2022
8	2500	Closed	Holstein-Friesian	Seasonal	103	26th Aug 2021 – 14th Aug 2021	7th Sep 2022 – 21st Sep 2022
9	1200	Open	Holstein-Friesian	Year-round	99	27th Jul 2021 – 14th Sep 2021	22nd Aug 2022 – 6th Oct 2022
10	2800	Closed	Holstein-Friesian	Seasonal	101	30th Aug 2021 – 6th Sep 2021	13th Aug 2022 – 10th Sep 2022
11	2800	Closed	Holstein-Friesian	Seasonal	102	20th Aug 2021 – 31st Aug 2021	8th Aug 2022 – 1st Sep 2022
12	650	Open	Holstein-Friesian	Year-round	103	21st Aug 2021 – 22nd Sep 2021	27th Aug 2022 – 8th Oct 2022

#: Operation types are classified into open and closed dairy herds.

Table S 4.2 A cross-tabulation of serological status against *Brucella* spp. at the initial test and reasons for follow-up losses.

Reasons	Number of Seropositive	Number of animals	Seroprevalence (%)
Still on the herd (unsampled in the second sampling)	8	18	44.4
Lameness	1	6	16.7
Low milk production	6	15	40.0
Mastitis	3	16	18.8
Aging	1	7	14.3
Reproductive disorder	4	12	33.3
Total	23	74	31.1

Table S 4.3 A table shows the longitudinal serological test results against *Brucella* spp., including follow-up and follow-up loss populations.

Group	1st test	2nd test	Number of animals
Follow-up group	Positive	Positive	125
	Positive	Negative	9
	Negative	Negative	822
	Negative	Positive	124
Follow-up lost	Positive		23
	Negative		51
Total number at 1st test	Positive		157
	Negative		997
Total number at 2nd test		Positive	249
		Negative	831

Figure S 4.1 Scatter plot for daily milk yield (kg per day) with days in milking in each *Brucella* exposure status

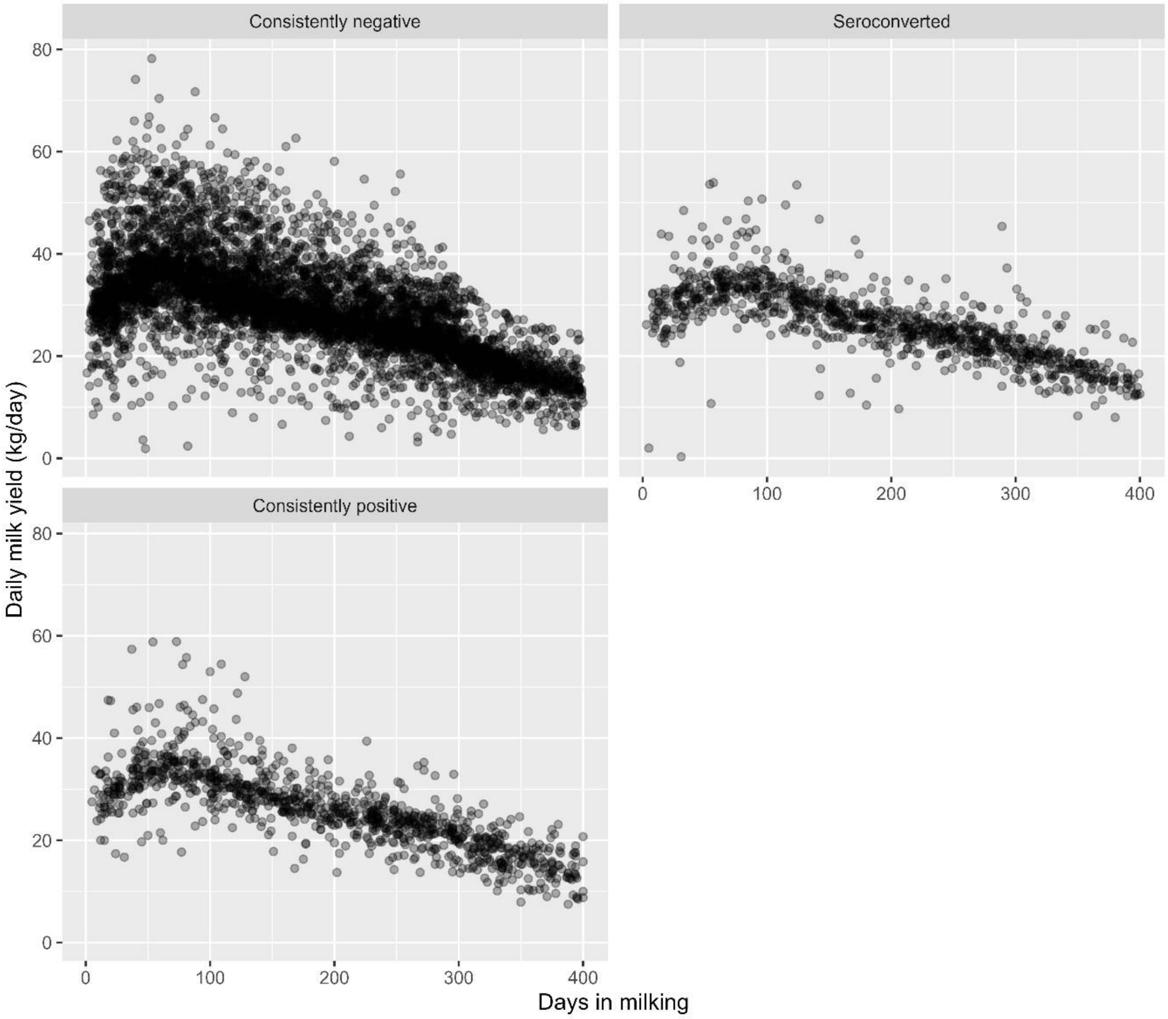
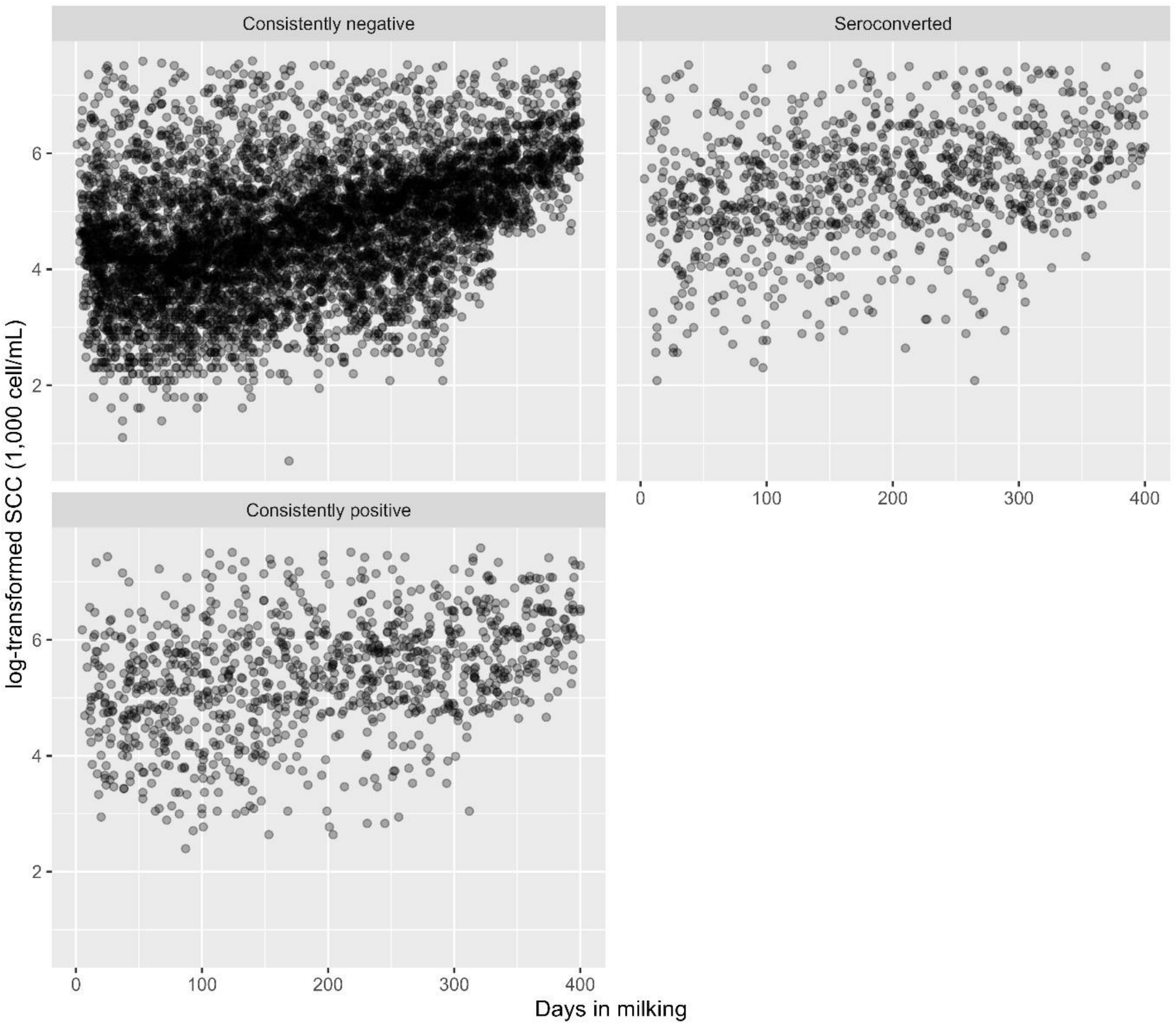


Figure S 4.2 Scatter plot for log-transformed somatic cell count (1000 cells per mL) with days in milking in each *Brucella* exposure status



Appendix 5

Table S 5.1 Sensitivity analysis explores the potential impact of changing input variables on the cost-benefit analysis.

Supplementary scenarios	Changed variable(s)	Alternative input parameters	Descriptions
Scenario sA	Apparent prevalence	1.0%	Lower prevalence
Scenario sB	Apparent prevalence	5.0%	Higher prevalence
Scenario sC	Sampling proportion	40%	10% decrease in sampling proportion for test-and-culling
Scenario sD	Sampling proportion	60%	10% increase in sampling proportion for test-and-culling
Scenario sE	Combined sensitivity Combined specificity	0.885 0.991	RBT-SAT is interpreted in parallel to increase sensitivity at the expense of reduced specificity
Scenario sF	Vaccine efficacy	Beta(5.28, 2.42)	weakly informative, 95% certainty > 0.4 at 0.751
Scenario sG	Vaccine efficacy	Beta(23.31, 8.40)	strongly informative, 95% certainty > 0.6 at 0.751
Scenario sH	Partial compensation for culled cows	50%	Half compensation
Scenario sI	Partial compensation for vaccine procurement	50%	Half compensation
Scenario sJ	Partial compensation for vaccine procurement and culled cows	50%	Half compensation
Scenario sK	No compensation for culled cows	0%	No compensation
Scenario sL	No compensation for vaccine procurement	0%	No compensation

Supplementary scenarios	**Changed variable(s)**	**Alternative input parameters**	**Descriptions**
Scenario sM	No compensation for vaccine procurement and culled cows	0%	No compensation