RESEARCH ARTICLE

Detection and analysis of *Mycoplasma bovis* infection in beef cattle in Ningxia Hui Autonomous Region of China

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Mycoplasma bovis* is a cell wall-free pathogen that mainly causes bovine pneumonia, arthritis, and mastitis. The world is facing challenges such as low vaccination rates and antibiotic resistance, urgently requiring new technologies for prevention, control, and treatment. *M. bovis* in Ningxia, China mostly causes pneumonia, which is related to transportation stress and high breeding density. This research investigated different scale cattle farms and individual farmers in five prefecture-level cities of Ningxia Hui Autonomous Region to explore the epidemic situation of *M. bovis* in beef cattle by observing the clinical symptoms of cattle and analyzing the pathological changes. A total of 491 samples were collected including lung and tracheal tissue samples from dead cattle and nasal swabs of cattle with respiratory symptoms to isolate and identify *M. bovis* and analyze the relationships between the positive rate and time, space, cattle age, season, and farm size. The results showed that there were significant regional differences in the prevalence of *M. bovis*. The highest positive rate was 29.59% in Wuzhong followed by Yinchuan, Guyuan, Zhongwei, and Shizuishan cities with the difference between Wuzhong and Guyuan and Shizuishan showing statistically significant ($P < 0.05$). The average positive rate of calves aged 1-3 months was 39.05%, which was significantly higher than that of calves aged 4-6 and 7-12 months ($P < 0.01$). The positive rates in autumn and winter were significantly higher than that in spring and summer ($P < 0.01$). The infection rate of large-scale farms was 24.88%, which was significantly higher than that of small-scale farms ($P < 0.01$). These results indicated that the prevalence of *M. bovis* might be affected by geography, breeding density, climate, and other factors. This study enriched the epidemiological data of *M. bovis* in cattle in Ningxia, China, clearly presented its prevalence in local beef cattle, and provided a basis for formulating effective prevention and control measures.

Keywords: *Mycoplasma bovis*; positive rate; epidemiology; beef cattle.

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Introduction

Mycoplasma bovis is a pathogenic microorganism that poses a great threat to the cattle breeding industry. It is not only the main pathogen of bovine respiratory diseases, but also one of the most important pathogens known to infect cattle [1]. *M. bovis* can not only cause *M.*

bovis pneumonia but also damage the function of the reproductive system. It is also the main pathogenic factor of arthritis [2], mastitis [3], tenosynovitis, conjunctivitis, and other diseases [4]. Since its first separation in the United States in 1961, it has become globally popular [5]. In Europe and North America, the respiratory and breast diseases caused by it have caused serious economic losses to animal husbandry [6].

M. bovis can infect cattle of all ages, but there are differences in susceptibility among different age groups. Among them, calves are the most susceptible to infection, and the morbidity and mortality are extremely high [7]. Clinical symptoms such as depression, dyspnea, cough, weakness, and elevated body temperature can occur in infected cattle at an early stage [2]. Infected cattle and carriers are the main source of transmission. Pathogens can be transmitted through respiratory secretions, milk, semen, and contaminated feed, litter, feces or aerosols [8]. High-density feeding in cattle barns can significantly increase the risk of disease outbreaks [9]. In addition, the flow of cattle promotes the spread of disease, and infected cattle can carry pathogens for a long time, resulting in repeated outbreaks. Because the pathogen is resistant to multiple antibiotics [10], and there is no effective vaccine in China [11], the prevention and control of *M. bovis* still faces great challenges. As a conditional pathogen, *M. bovis* is ubiquitous in cattle and causes disease only under specific conditions such as transport stress, regrouping, or cold environments with impaired immunity [7], which highlights the need to develop a comprehensive long-term strategy, focusing on optimizing feeding management and continuous health monitoring. Liu *et al*. performed multi-gene sequence typing on milk and breast samples from 23 Chinese dairy farms in 2018 and 2019 and detected 42.30% and 8.70% positive rates of *M. bovis*, respectively [12]. Niu *et al*. found a 48.70% seropositive rate in Tibetan yaks from 2019 to 2020 [13]. These findings indicate that *M. bovis* is endemic in China, and its widespread prevalence may be related to diverse breeding environments, feeding methods, and regional climatic conditions.

This study investigated the prevalence of *M. bovis* in beef cattle in Ningxia Hui Autonomous Region of China using nasal swabs and diseased lung tissue and trachea samples collected from different scale cattle farms and free-range households in five cities of Ningxia, China including Guyuan, Zhongwei, Yinchuan, Wuzhong, and Shizuishan through polymerase chain reaction (PCR) technology. The results of this study provided scientific basis for epidemic prevention and control of *M. bovis* in beef cattle and helped the sustainable development of local beef industry.

Materials and methods

Sample collection

A total of 491 samples including 404 nasal swab samples, 69 lung tissue samples, and 18 trachea samples were collected from beef cattle farms of different sizes and small family ranches in five prefecture-level cities of Guyuan, Zhongwei, Yinchuan, Wuzhong, and Shizuishan in Ningxia Hui Autonomous Region of China. The samples were divided into two categories as the lungs or tracheas of cattle died of respiratory symptoms and nasal swab samples from live cattle with significant clinical symptoms of respiratory tract. Among them, 96 nasal swabs, 16 lung tissues, and 9 tracheas were collected in Yinchuan City. 15 nasal swabs and 6 lung tissues were collected in Zhongwei City. A total of 140 nasal swabs, 25 lung tissues, and 4 tracheas were collected from Wuzhong City, while a total of 141 nasal swabs, 14 lung tissues, and 5 tracheas were collected in Guyuan City. A total of 12 nasal swabs and 8 lung tissues were collected in Shizuishan City. All procedures of this research were approved by the Scientific and Technological Ethics Committee of Ningxia Academy of Agricultural and Forestry Sciences (Yinchuan, Ningxia Hui Autonomous Region, China).

Primer synthesis

The uvrC-specific primers of *M. bovis* were designed according to the research of Guo *et al*. [14]. The sequences of primers were uvrC forward primer (5'-GAA TTC AAT GTG TCT ACT AGT CCT GG-3') and uvrC reverse primer (5'-AAG CTT AGC GTC AGA TTT TTG CAT A-3') with a 1,620 bp potential product at 55°C annealing temperature. All primers were synthesized by Shanghai Shenggong Biotechnology Co., Ltd., Shanghai, China).

Pathogen isolation and purification

Nasal swabs and tissue samples were inoculated in pleural pneumonia-like microbial (PPLO) agar (Beckman Coulter, Franklin Lex, New Jersey, USA) containing 20% horse serum and cultured at 37°C for 24 - 48 hours before the *Mycoplasma* colonies were observed under a microscope. The selected colonies were transferred to PPLO broth and cultured at 37°C, 220 rpm, for 24 - 48 hours.

DNA extraction and PCR amplification

2 mL of PPLO broth culture were taken for genomic DNA extraction by using a bacterial genomic dna extraction kit (Tiangen, Beijing, China) following manufacturer's instructions. The extracted genomic DNA was subjected to PCR amplification with sterile water as a negative control and the verified *M. bovis* (Institute of Animal Science, Yinchuan, Ningxia, China) as a positive control. The 50 µL PCR reaction mixture contained 5 µL of 10× PCR buffer, 4 µL of dNTP mixture, 0.25 µL of rTaq polymerase, 1 µL of each primer, 1 µL of DNA template, and 37.75 µL deionized water. The PCR reaction was conducted using Thermo Fisher Scientific, MiniAmp™ Plus Thermal Cycler (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) under the program of 95°C for 2 min followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, and finally 72°C extension for 5 min. The PCR products were sent to Shanghai Biotechnology Co., Ltd., (Shanghai, China) for subsequent DNA sequencing and analysis.

Data statistical analysis

The sample data were sorted out by using Microsoft Excel (Microsoft, Redmond, Washington, USA) and statistically analyzed by using SPSS 20.0 software (IBM, Armonk, New York, USA) for the data differences of year, region, age, season, and farm size through Chi-square test. The results were visualized by using GraphPad Prism 8.0.2.263 (www.garphpad.com).

Results

Detection of *M. bovis* in different regions of Ningxia Hui Autonomous Region

Among 491 collected samples, 109 samples were detected to have *M. bovis*. Therefore, the positive rate of *M. bovis* in Ningxia was 22.19%. The detection results showed that the detection rates of *M. bovis* in five prefecture-level cities were 20.66% (25/121) in Yinchuan, 9.52% (2/21) in Zhongwei, 29.59% (50/169) in Wuzhong, 19.38% (31/160) in Guyuan, and 5.00% (1/20) in Shizuishan with Wuzhong being the highest and Shizuishan being the lowest. The difference analysis showed that there was a statistically significant difference in the detection rate of *M. bovis* between Wuzhong and Guyuan and Shizuishan ($P < 0.05$) (Figure 1).

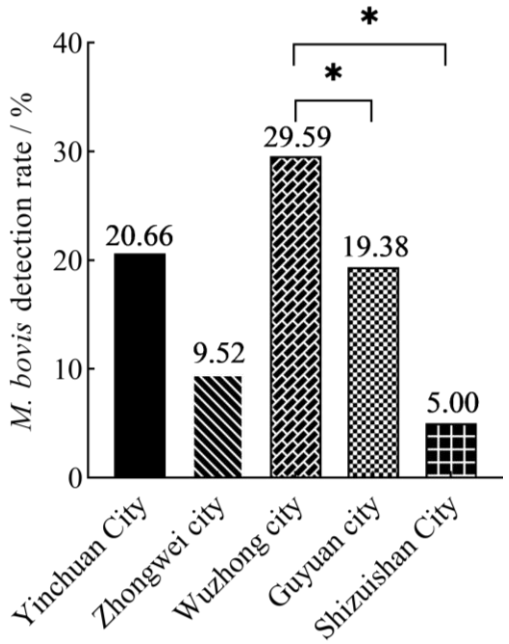

Figure 1. Comparative analysis of *M. bovis* detection rates across different regions in Ningxia, China. *: $P < 0.05$.

Detection of *M. bovis* in different age groups

Statistical analysis of the detection rate of *M. bovis* in different age groups showed significant differences with 39.05% (66/169) in 1 - 3 months, 16.26 % (20/123) in 4 - 6 months, 4.62% (6/130) in 7 - 12 months, and 24.64% (17/69) in 2 - 3 years. The detection rate of *M. bovis* in 1 - 3 months old was the highest, while the detection rate of *M. bovis* in 7 - 12 months old was the lowest. The positive rate of *M. bovis* in 1 - 3 months old was significantly higher than that in 4 - 6 months old and 7 - 10 months old ($P < 0.01$). The positive rate of *M. bovis* in 2 - 3 years old was significantly different from that in 7 - 10 months old ($P < 0.05$). There was no significant difference between the other age groups (Figure 2).

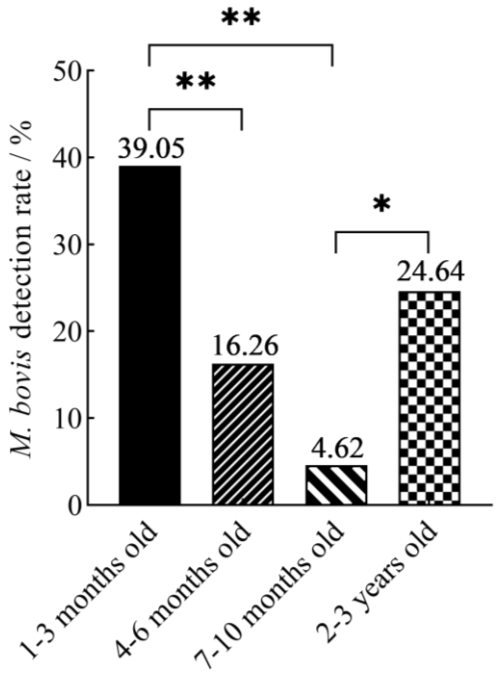

Figure 2. Comparative analysis of *M. bovis* detection rates among different age groups in Ningxia region. *: $P < 0.05$. **: $P < 0.01$.

Seasonal changes in the detection rate of *M. bovis*

Ambient temperature has been shown to have a significant effect on pathogen prevalence. The results showed that the detection rates of *M. bovis* were 37.97% (30/79), 16.67% (40/240), 13.28% (17/128), and 50.00% (22/44) in spring, summer, autumn, and winter, respectively. The detection rates of *M. bovis* in spring and winter were much higher than that in summer and autumn. Statistical comparison showed that the detection rate of *M. bovis* in winter was significantly higher than that in summer and autumn ($P < 0.01$), and the detection rate of *M. bovis* in spring was also significantly higher than that in summer and autumn ($P < 0.01$). There was no significant difference between the other seasons (Figure 3). The prevalence of pathogens in cold season of winter and spring was significantly higher than that in warm seasons of summer and autumn.

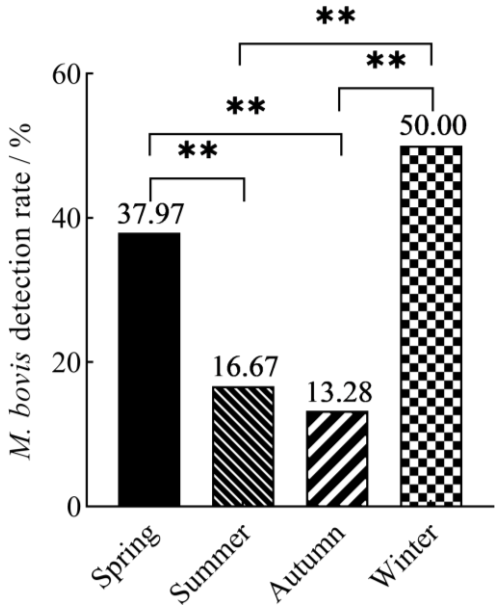

Figure 3. Differential analysis of seasonal variation in *M. bovis* detection rates in Ningxia region. **: $P < 0.01$.

Detection of *M. bovis* in different scale farms

The impact of farm size and stocking density on the incidence of respiratory diseases was evaluated based on herd size classification with the large farms defined as ≥ 100 cattle and small farms defined as < 100 cattle. The results showed that the detection rate of *M. bovis* in large pastures was 24.88% (103/414), while the detection rate of *M. bovis* in small farms was 7.79% (6/77). The positive rate of *M. bovis* in large pastures was three times more than that of

small farms. The comparative analysis showed that there was a significant difference in the detection rate between the two culture systems ($P < 0.01$) (Figure 4).

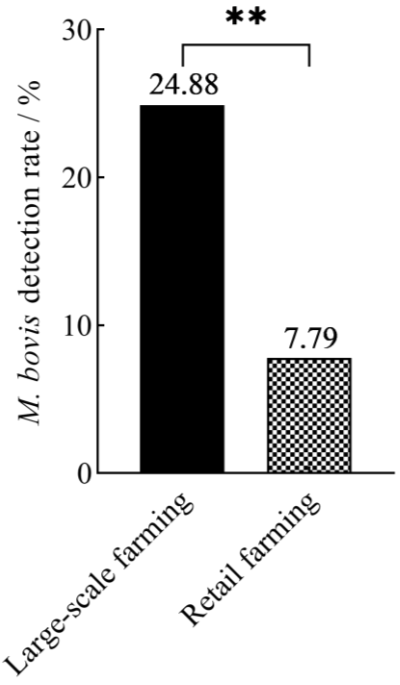

Figure 4. Comparative analysis of *M. bovis* detection rates between different farm scales in Ningxia region. **: $P < 0.01$.

Discussion

In previous studies, Guo *et al*. reported the positive rates of *M. bovis* antibody in Ningxia serological survey in 2020 and 2021 as 29.39% and 37.58%, respectively [15]. Further, Niu *et al*. found 48.70% serum positive rate in Tibetan yaks from 2019 to 2020 [13]. In this study, the epidemiological investigation of *M. bovis* in beef cattle in Ningxia Hui Autonomous Region of China showed that the overall positive rate was 22.19 %. There were changes in time. The low detection rate of *M. bovis* in this study indicated a downward trend in Ningxia, China since 2021, which might be attributed to the strengthening of respiratory disease management measures including the optimization of immunization programs and the improvement of disinfection measures in local cattle farms. The specific analysis of the positive rate of *M. bovis* and age showed that the infection rate of calves aged 1 - 3 months was the highest with the mean value of 39.29% and the peak at 3 months old reaching 42.03%, while the infection rate of calves aged 7 - 12 months was the lowest with the average of 3.85% and the lowest point of 3.23% at 7 months of age. The results demonstrated significant difference. This inverse relationship between age and infection rate was consistent with the results of Gogoi-Tiwari *et al*. who reported that the positive rate of calf serum was 61%, and that of adult cattle was 42.5% [9], Gabinaitiene *et al*. who observed that the positive rate decreased from 22.9 % to 11.4 % in the ages of ≤ 3 months and 17 months, respectively [16], Li *et al*. who found that the antibody positive rate of calves less than 6 months old was much higher than that of young cattle and adult cattle in the serological detection of *M. bovis* in four provinces in western and northern China [17]. Further, Researchers analyzed the detection rate of *M. bovis* in beef cattle at different growth stages and found that the detection rate of *M. bovis* in lactation period was significantly higher than that in breeding period, and there was no significant difference in the detection rate between lactation period and mature period [18]. The high susceptibility of calves might be due to the vertical transmission of vaginal secretions/milk of infected cows and their immature immune defense mechanisms [19]. Geographical spatial analysis revealed that the infection rate of *M. bovis* had significant regional differences in Ningxia Hui Autonomous Region with the infection rate of *M. bovis* in Wuzhong as the highest one (29.59%), while that in Shizuishan was only 5.00%. This regional distribution might be related to the dense cattle trading network in Wuzhong and the difference in industrial dominance in Shizuishan. Seasonal regularity showed that the incidences of winter and spring were high, which were consistent with the report of Wen *et al*. [20]. It might be affected by the enhanced survival ability of pathogens in cold and dry environments and cold-induced immunosuppression. The infection rate of *M. bovis* in large farms was 3.19 times higher than that in small farms ($P < 0.01$), highlighting the risk

of density-dependent transmission. These research findings collectively revealed various epidemiological characteristics of *M. bovis* including the interplay of management practices, environmental stressors, and host factors, all of which influenced the dynamics of the disease. As an important cattle breeding area in China, the Ningxia authorities should attach great importance to *Mycoplasma bovis* disease. The disease publicity should be strengthened to make farmers understand the hazards and prevention methods, strengthen prevention and control measures to better cope with the epidemic of *M. bovis*, and ensure the sustainable development of China's cattle industry.

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