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RESEARCH ARTICLE

The Impact of BVDV Infection on the Immune Efficacy of *Brucella abortus* A19 Vaccine

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Abstract

As *Bovine Viral Diarrhea Virus* (BVDV) is widespread in cattle and often causes immunosuppression, it is essential to evaluate whether it interferes with the effectiveness of widely used live vaccines such as *Brucella abortus* A19. This study evaluated the impact of BVDV infection on the immunoprotective efficacy of the *B. abortus* A19 vaccine using co-infection models in mice and RAW264.7 macrophages. The animal experiments revealed that co-infection led to significantly higher splenic bacterial loads of *B. abortus* A19 on days 14, 42, and 56 post-infection (pi), along with an increased spleen index on days 14, 28, 42, and 56 pi, compared to *B. abortus* A19 single-infection group. Results from the cell model demonstrated that co-infection enhanced intracellular survival of *B. abortus* A19 and significantly upregulated IL-10 mRNA and protein levels while suppressing TNF-α mRNA. In contrast, *B. abortus* A19 single-infection group induced higher expression of IL-12 and IFN-γ compared to BVDV single-infection group or co-infected groups. In conclusion, BVDV infection compromises the protective efficacy of the *B. abortus* A19 vaccine by enhancing the intracellular survival of *B. abortus* A19 in macrophages and dysregulating the expression of immune-related cytokines. These findings highlight the importance of BVDV control in vaccination programs and provide new insights into the mechanisms underlying vaccine failure under co-infection conditions.

Keywords: Coinfection, Cytokines, Immunity, Intracellular survivaldynamics

Introduction

Bovine viral diarrhea mucosal disease (BVD-MD) is an infectious disease that affects the global distribution of cattle, caused by BVDV, which is a single stranded RNA virus. In China, BVDV was first isolated from aborted bovine fetuses in 1980 [1]. So far, three different BVDV genotypes have been identified, namely BVDV1, BVDV2, and BVDV3. According to the phenotypic characteristics in cell culture, BVDV strains within each genotype are further classified into two biotypes: cytopathic (CP) and noncytopathic (NCP) [2]. Cattle are the natural host of BVDV, and infections typically manifest as clinical symptoms including fever, diarrhea, miscarriage, stillbirth, and mummified fetuses [3]. NCP type BVDV can cause persistent infection (PI) in cattle through fetal infection during early pregnancy [4]. Although PI cows do not have obvious clinical symptoms, they exhibit a range of adverse features, including impaired growth performance, reduced milk production, decreased survival rates, increased susceptibility to other pathogens, and persistent viral shedding, leading to the continuous circulation and transmission of BVDV in the herd, causing huge economic losses. [5-7].

Brucella is a globally prevalent zoonotic infectious disease caused by bacteria of the *Brucella* genus [8]. *Brucella* has a wide range of hosts, including ungulates, carnivores, rodents, primates, and marine mammals. Infection with this pathogen can induce a series of pathological conditions in the affected host, such as miscarriage, placental inflammation, orchitis, and epididymitis [9]. Currently, vaccination is considered the most cost-effective and effective strategy for preventing and controlling brucellosis [10]. In China, seven *Brucella* vaccines have been officially approved for commercial use, classified according to their bacterial sources: sheep *Brucella* derivative vaccines (strains M5/M5-90, M5-90 Δ 26, *Rev. 1*, and BA0711), pig *Brucella* derivative vaccines (strain S2), and *Brucella abortus* derivative vaccines (strains A19 and A19 Δ *VirB12*) [11]. Among these approved vaccines, *B. abortus* A19 strains are the most widely used in China's livestock industry. Their application can effectively reduce the miscarriage rate of infected animals, thereby alleviating the economic losses of livestock farmers [12].

The success of vaccine immunization largely depends on the host's robust immune system, which is the physiological basis for producing specific antibodies and cellular immune responses. Acute BVDV infection can lead to depletion of lymphocytes in lymphoid organs such as thymus, spleen, and lymph nodes, resulting in immune dysfunction [13,14]. Wang Chuanfeng et al.[15] found that the swine fever vaccine contaminated with BVDV leads to immune failure against swine fever, because BVDV stimulates the production of large amounts of BVDV antibodies in vaccinated pigs while also inhibiting the production of swine fever antibodies. In addition, increasing evidence suggests that BVDV exerts immunomodulatory effects by disrupting immune cells and promoting their migration to tissues where viral replication occurs. These processes lead to a decrease in lymphocytes and neutrophils, ultimately making infected cows more susceptible to secondary infections from other pathogens [16,17]. Therefore, if cows are infected with BVDV before vaccination with the *B. abortus* A19 vaccine, there is a high probability that the immune protection efficiency of the A19 attenuated vaccine will be reduced.

So far, there is limited literature on the co-infection of BVDV and the *B. abortus* A19 vaccine, and information on the interaction between BVDV infection and *B. abortus* A19 vaccination is still limited. This study established a C57BL/6J mouse model co-infected with BVDV and *B. abortus* A19 (BVDV+A19 co-infected). Subsequently, systematic analysis and comparison were conducted on laboratory test data from three experimental groups, namely BVDV single-infection group, *B. abortus* A19 single-infection group, and BVDV+A19 co-infection group. These results provide key experimental evidence for the observed immune failure of BVDV epidemic bovine *Brucella* vaccine in clinical practice.

Material and Methods

Ethical Statement

This study was approved by the Biology Ethics Committee of Shihezi University (Approval no: A2025-1073, Date: October 2025).

Bacterial Strains, BVDV Strain, Cells, Animals, and Culture Conditions

The *B. abortus* A19 vaccine strain was from Xinjiang Center for Disease Control and Prevention. Strains stored at -80°C were inoculated onto BBL™ *Brucella* Agar (BD, Shanghai, China) and incubated at 37°C for 72 h. Single colonies were isolated and propagated in BBL™ *Brucella* Broth (BD, Shanghai, China) at 37°C for 24 h. Bacterial cultures were harvested for subsequent experimental procedures. The CP-type BVDV strain was generously provided by Professor Qiang Fu from Xinjiang Agricultural University. RAW264.7 cells (maintained in our laboratory) were cultured in DMEM medium supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Cells in the logarithmic growth phase were seeded into 6-well plates (NEST Biotechnology, Jiangsu, China) at a density of 1×10^6 cells/well and allowed to adhere for 24 h prior to experimentation. Six-week-old female C57BL/6J mice were obtained from Henan Skebes Biotechnology Co., Ltd. and housed in the Animal Hospital.

Establishment of a Mouse Model of Coinfection

A total of 100 six-week-old mice were randomly divided into four groups, with 25 mice in each group. Group 1 served as the control group, Group 2 as the BVDV single-infection group, Group 3 as the *B. abortus* A19 single-infection group, and Group 4 as the BVDV+A19 co-infected group. The four groups were intraperitoneally injected with 600 μL of 1640 medium, BVDV, 1640 medium, and BVDV, respectively, in sequence. After 14 days of feeding, the four groups were intraperitoneally injected with 200 μL of 1640 medium, 1640 medium, *B. abortus* A19 vaccine strain, and *B. abortus* A19 vaccine strain, respectively, the colony-forming unit (CFU) of the infected *B. abortus* A19 was 5×10^5 CFU/mL. After another 56 days of feeding, each group was intraperitoneally injected with 200 μL of 1640 medium, 1640 medium, *B. abortus* A19 vaccine strain, and *B. abortus* A19 vaccine strain, respectively, with the CFU of the infected *B. abortus* A19 being 1.5×10^6 CFU/mL. Mice in the four groups were reared in isolation in separate rooms with a clean environment, under completely identical feeding conditions, and provided with sufficient rodent food and water. The infection procedure of the mice has been shown *(Fig. 1)*.

Determination of Spleen Index and Splenic Bacterial Load in Mice

A mouse model of BVDV+A19 co-infection was established. On days 14, 28, 42, and 56 post-modeling, mice were weighed and subjected to aseptic dissection. The spleens of the mice were then weighed, and the spleen index was calculated based on the recorded data. Subsequently, each spleen was placed into a clean EP tube supplemented with 500 μL of 1x phosphate-buffered saline (PBS, Phosphate Buffered Saline). After grinding the spleen, 10-fold serial dilutions of the homogenate

Fig 1. Process diagram for intraperitoneal injection infection of BVDV and the B. abortus A19 in mice

were prepared. The diluted samples were spread onto the appropriate medium, followed by incubation for 3-4 days to perform colony counting. The splenic bacterial load of each mouse was calculated using the formula: Bacterial load = Average number of colonies × Homogenate volume x Dilution factor/Spleen weight. At each time point, 3 mice were included in each group for detection.

Establishment of a RAW264.7 Cell Model of Coinfection

RAW264.7 cells were thawed and seeded into 10 cm culture dishes. When the cell confluency reached 70-80%, the cells were inoculated with BVDV solution at a multiplicity of infection (MOI) of 1, followed by static adsorption for 2 h. After washing with PBS for 3 times, complete medium was added to the dishes for 24 h of culture. The cells were then isolated and equally divided into two aliquots: one aliquot was passaged into new culture dishes for continuous culture, the other aliquot was lysed with TRIzon reagent and stored at −80°C for subsequent RNA extraction. This process was repeated until the 10th passage (P1–P10). Total RNA was extracted from cells of each passage, and absolute quantitative real-time polymerase chain reaction (qPCR) was performed to determine the BVDV copy number. The complete list of primers used in this study has been shown *(Table 1)*.

Detection of Cell Viability by CCK-8 Assay

RAW264.7 cells in the logarithmic growth phase were seeded into a 96-well plate (NEST Biotechnology, Jiangsu, China) at a density of 50.000 cells per well. After the cells had adhered to the plate, cell models of single infection with BVDV, single infection with the *B. abortus* A19, and co-infection with BVDV+A19 were established respectively. The constructed cell models were statically cultured for 24 h. Subsequently, 10 µL of CCK-8 detection reagent was added to each well. After incubation for 2 h in the dark, the optical density (OD) value at a wavelength of 450 nm was measured. Cell viability was then calculated based on the measured OD values.

Detection of Cytokine mRNA and Protein Levels in the Coinfected Cell Model

RAW264.7 cells were seeded into 6-well plates (NEST Biotechnology, Jiangsu, China). When the cell confluency reached 80%, cell models were established, including those with single infection of BVDV, single infection of the *B. abortus* A19, and co-infection of BVDV+A19. At 0 h, 12 h, and 24 h pi, total RNA was extracted from the cells, and cell supernatant samples were isolated. These samples were used for the detection of cytokine mRNA levels (from RNA) and cytokine protein levels (from cell supernatants), respectively. The complete list of primers used in quantitative real-time polymerase chain reaction (qRT-PCR) has been shown *(Table 2)*.

Table 1. *BVDV copy number absolute detection primers*

Primers	5'-3'Squence
5'-UTR1	GCTAGCCATGCCCTTAGTAGG
5'-UTR2	TCCATGTGCCATGTACAGC

Detection of Intracellular Survival Ability of A19

RAW264.7 cells were seeded into 6-well plates (NEST Biotechnology, Jiangsu, China). When the cell confluency reached 70-80%, the cells were inoculated with BVDV solution at a MOI of 1, followed by static adsorption for 2 h. Subsequently, the cells were washed 3 times with 1xPBS, and complete medium was added for static culture for 24 h. After this period, the cells were infected with the *B. abortus* A19 strain. At 0 h and 24 h pi, the cells were lysed with 0.1% Triton X-100. The cell lysate was subjected to 10-fold serial dilutions, and the diluted samples were spread onto the appropriate medium. After incubation for 72 h, colony counting was performed, and the results were recorded.

Statistical Analysis

All results of data analysis are presented as the mean ± standard deviation (SD). Data analysis was performed on independent samples using the t-test. Differences were considered as significant when P≤0.05. Statistical analysis was performed using GraphPad Prism 9 software (GraphPad, Inc., La Jolla, CA, USA).

Results

BVDV Infection Enhances the Survival and Proliferation Capacities of *B. abortus* A19 Vaccine Strain in Mouse Spleens

Following the successful establishment of intraperitoneal infections with the BVDV strain and the *B. abortus* A19 strain, mice in the BVDV single-infection group, the *B.*

Table 2. *Complete primer list for qRT-PCR*

Primers	5'-3'Squence
*Il10*1	GCTCTTACTGACTGGCATGAG
*Il10*2	CGCAGCTCTAGGAGCATGTG
*Il12b*1	TGGTTTGCCATCGTTTTGCTG
*Il12b*2	ACAGGTGAGGTTCACTGTTTCT
*Ifng*1	ATGAACGCTACACACTGCATC
*Ifng*2	CCATCCTTTTGCCAGTTCCTC
*Tnfa*1	GACGTGGAACTGGCAGAAGAG
*Tnfa*2	TTGGTGGTTTGTGAGTGTGAG
*β-actin*1	TGCTATGTTGCTCTAGACTTCG
*β-actin*2	GTTGGCATAGAGGTCTTTACGG

abortus A19 single-infection group, and BVDV+A19 co-infection group all exhibited clinical signs including lethargy and ruffled fur. Mice were euthanized and dissected on days 14, 28, 42, and 56 pi. Compared with the control group and BVDV single-infection group, mice in the *B. abortus* A19 single-infection group and BVDV+A19 co-infection group showed significant splenomegaly. Notably, the splenic index of mice in the BVDV+A19 co-infection group was significantly higher than that in the control group, BVDV single-infection group, and the *B. abortus* A19 single-infection group at all tested time points (on days 14, 28, 42, and 56 pi) ($P \leq 0.01$) *(Fig. 2)*. On days 14, 42, and 56 pi, the bacterial load of the *B. abortus* A19 in the spleens of mice in the co-infection group was significantly higher than that in the *B. abortus* A19 single-infection group ($P \leq 0.05$) *(Fig. 3)*. Collectively, these results demonstrate that co-infection with BVDV and the *B. abortus* A19 strain significantly exacerbates pathological damage to the mouse spleen and promotes the survival and proliferation of the *B. abortus* A19 in the spleen.

Establishment of a BVDV-Infected RAW264.7 Macrophage Model

Total RNA was extracted from passages 1 to 10 of RAW264.7 macrophages infected with BVDV. After reverse transcription, the copy number of BVDV was detected by qRT-PCR. the logarithmic (lg) value of BVDV copy number in RAW264.7 cells from passage 1 to passage 6 was stably maintained between 5 and 6, showing a relatively steady level. Starting from passage 7, the lg value of BVDV copy number decreased slightly to approximately 4.25, from passage 8 to passage 10, it remained between 4 and 5, with a detectable amount of BVDV copy number. In conclusion, BVDV can stably persist in RAW264.7 cells, indicating the successful establishment of the BVDV-infected RAW264.7 macrophage model *(Fig. 4)*.

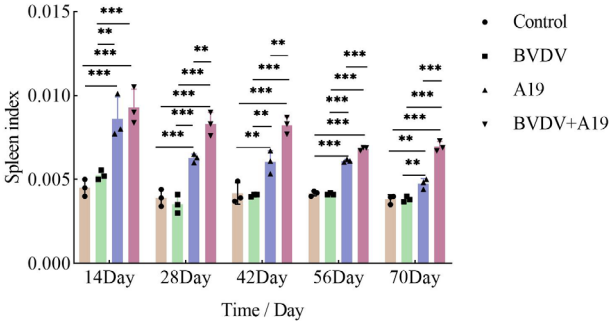

Fig 2. Calculation results of mouse spleen index

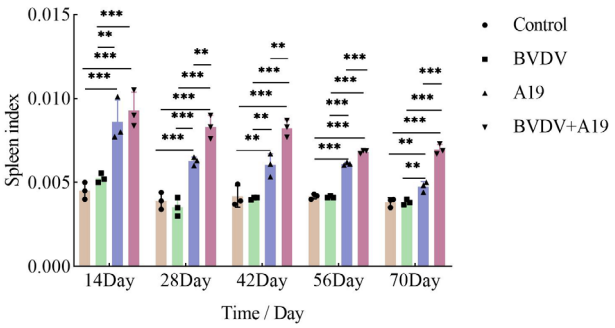

Fig 3. Determination results of bacterial load in mouse spleen

BVDV and the *B. abortus* A19 Co-Infection Reduces the Viability of RAW264.7 Macrophages

To investigate the effect of BVDV infection on the viability of RAW264.7 cells, the cell viability of the blank control group, BVDV single-infection group, the *B. abortus* A19 single-infection group, and BVDV+A19 co-infection group was detected at 24 h pi using the Cell Counting Kit-8 (CCK-8) assay. Compared with the control group, both single-infection groups induced an extremely significant decrease in the viability of RAW264.7 cells ($P<0.001$). Notably, the cell viability in the BVDV+A19 co-infection group decreased more remarkably: its viability was not only extremely significantly lower than that of the control group ($P<0.001$) but also extremely significantly lower than that of either single-infection group ($P<0.001$). These results indicate that BVDV and the *B. abortus* A19 exert a synergistic effect in inhibiting cell viability *(Fig. 5)*.

BVDV Infection Disrupts the Cytokine Secretion Balance in RAW264.7 Cells Induced by the *B. abortus* A19 Vaccine Strain

To investigate the effect of BVDV infection on the immune response of RAW264.7 cells induced by the *B. abortus* A19, the mRNA and protein expression levels of interleukin-10 (IL-10) and interleukin-12 (IL-12) in RAW264.7 cells were detected at 24 h pi using qRT-PCR and enzyme-linked immunosorbent assay (ELISA), respectively.

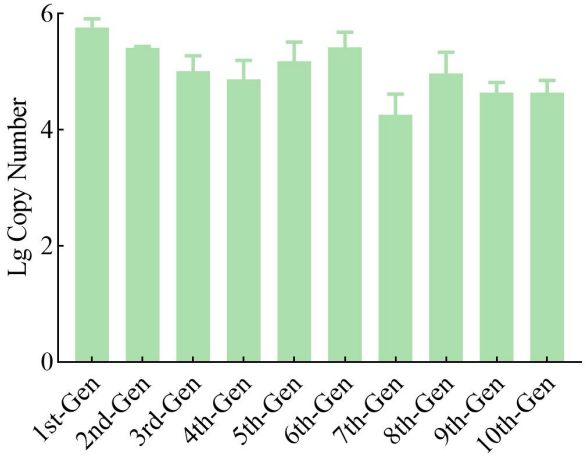

Fig 4. BVDV copy number in RAW264.7 cells upon serial passaging

Compared with the Control group, the relative mRNA expression levels of IL-10 in both the BVDV single-infection group and the BVDV+A19 co-infection group were significantly upregulated (P<0.01). Additionally, the relative IL-10 mRNA expression level in the *B. abortus* A19 single-infection group was significantly lower than that in the BVDV single-infection group and the BVDV+A19 co-infection group (P<0.01) *(Fig. 6-A)*. At the protein level, the IL-10 concentration in the *B. abortus* A19 single-infection group was significantly downregulated compared with the BVDV+A19 co-infection group (P<0.001) *(Fig. 6-B)*. Regarding tumor necrosis factor-α (TNF-α), the relative mRNA expression levels in the BVDV single-infection group and the BVDV+A19 co-infection group were both significantly lower than that in the *B. abortus* A19 single-infection group (P<0.001) *(Fig. 6-E)*.

For IL-12, the relative mRNA expression level in the *B. abortus* A19 single-infection group was significantly higher than those in the BVDV single-infection group and the BVDV+A19 co-infection group (P<0.05) *(Fig. 6-C)*. Consistently, the IL-12 protein concentration in the *B. abortus* A19 single-infection group was significantly upregulated compared with the BVDV single-infection group and the BVDV+A19 co-infection group (P<0.001) *(Fig. 6-D)*. Furthermore, the relative mRNA expression levels of interferon-γ (IFN-γ) in the BVDV single-infection group and the BVDV+A19 co-infection group were significantly lower than that in the *B. abortus* A19 single-infection group (P<0.001) *(Fig. 6-F)*.

Collectively, these data demonstrate that BVDV infection can effectively inhibit the expression and secretion of proinflammatory cytokines in RAW264.7 cells, suggesting that BVDV may evade the host innate immune response by impairing the immune activation function of macrophages.

BVDV Infection Enhances the Intracellular Survival of the *B. abortus* A19 Strain in RAW264.7 Cells

To investigate the effect of BVDV infection on the intracellular survival of the *B. abortus* A19 strain in RAW264.7 cells, colony counting was performed to determine the number of intracellular colonies of the *B. abortus* A19 (MOI=100) at 0 and 24 h pi in RAW264.7 cells. At both 0 h pi and 24 h pi, the number of intracellular the *B. abortus* A19 colonies in the BVDV+A19 co-infection group was significantly higher than that in the *B. abortus* A19 single-infection group (P<0.01) *(Fig. 7)*. These results indicate that BVDV may regulate the immune function of cells, thereby creating a favorable environment for the survival and proliferation of the *B. abortus* A19 vaccine strain.

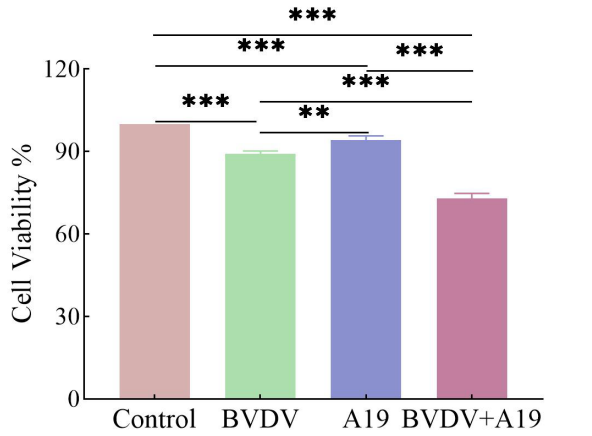

Fig 5. Comparative analysis of cell viability across different treatment groups

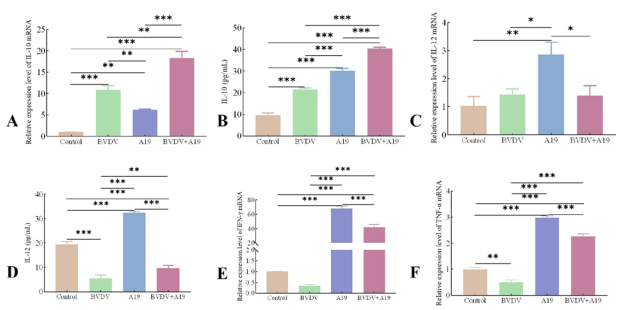

Fig 6. Analysis of mRNA and protein expression levels of cytokines under different treatment conditions. A-E represent the relative expression levels of IL-10 mRNA, protein, IL-12 mRNA, protein, IFN-γ mRNA, and TNF-α mRNA, respectively

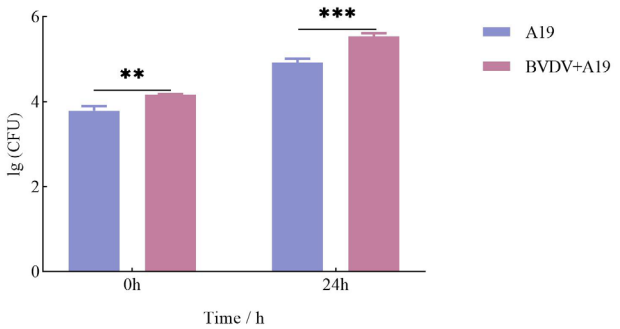

Fig 7. Intracellular survival status of the *B. abortus* A19 and BVDV+A19

Discussion

This study established a co infection model between bovine viral diarrhea virus (BVDV) and *Brucella* vaccine strain A19, and for the first time confirmed that BVDV infection can significantly reduce the immune-protective efficacy of *Brucella* vaccine. This discovery not only reveals the cross-pathogen interference mechanism of the virus on the immune response of bacterial vaccines, but also provides an important explanation for the previously overlooked

phenomenon of inadequate vaccine protection in current brucellosis prevention and control, that is, the widespread prevalence of BVDV may play a key role in it. Therefore, the results of this study further indicate that incorporating BVDV monitoring and control into the overall prevention and control strategy in brucellosis purification planning is not an auxiliary measure, but a necessary prerequisite for achieving effective immunization and ultimately achieving disease eradication goals.

In vivo infection data revealed that, compared with the *B. abortus* A19 single-infection group, mice in the BVDV+A19 co-infection group exhibited significantly higher bacterial loads in the spleen at all tested time points (on days 14, 42, and 56 pi). The spleen is a key immune organ where *Brucella* colonizes and elicits immune responses, and its bacterial load directly reflects the host's ability to clear the pathogen [18]. The sustained elevation of splenic bacterial load in the co-infection group may be attributed to BVDV-mediated suppression of the host immune system: BVDV can directly infect and damage lymphocytes and macrophages, leading to immune cell dysfunction and reduced cell numbers. This impairment severely diminishes the ability of mice to clear the *B. abortus* A19 strain, thereby promoting the survival and proliferation of the *B. abortus* A19 [19, 20].

Additionally, the splenic index of mice in the co-infection group was significantly higher than that in the *B. abortus* A19 single-infection group throughout the entire infection cycle (on days 14, 28, 42, and 56 pi). This elevated splenic index is more likely a consequence of BVDV infection disrupting the host's normal immune regulatory balance, triggering an excessive yet ineffective inflammatory response that ultimately results in lymphocyte necrosis and direct impairment of splenic function. Consistent with this, Liu et al.[21] also confirmed that lymphocyte degeneration and necrosis occur in the spleen of mice in the late stage of BVDV infection.

Together, the observed persistence of high bacterial loads alongside splenic enlargement in co-infected mice suggests that BVDV infection substantially compromises the host's ability to control *B. abortus* A19. This impaired clearance may reflect a dysregulated immune state that permits prolonged bacterial survival *in vivo*. These *in vivo* co-infection phenotypes establish a foundation for further investigation into the cellular and molecular mechanisms involved.

To elucidate the cellular basis of the impaired bacterial clearance observed *in vivo,* we employed the RAW264.7 macrophage model. Consistent with the splenic bacterial load data, BVDV infection significantly enhanced the intracellular survival of *B. abortus* A19 in macrophages. This suggests that BVDV alters the host cell microenvironment in a way that favors bacterial persistence. A potential explanation is that BVDV infection activates the IFN-β pathway in RAW264.7 cells in the early stage to exert antiviral effects, however, in the late stage of infection, the upregulation of SOCS1 (a negative regulator of IFN-β) weakens the sustained antiviral state of the cells, thereby indirectly impairing their bacteriostatic capacity [22]. Alternatively, BVDV infection of host cells may activate the NLRP3 inflammasome, triggering pyroptosis and subsequent cell death, which directly reduces the bacteriostatic capacity of host cells [23].

Moreover, cytokines, as key messenger molecules for intercellular communication, are primarily involved in the regulation of immune homeostasis and inflammatory responses [24]. IL-10 acts on macrophages themselves to inhibit the production of proinflammatory cytokines (e.g., TNF-α, IL-1β), reduce antigen-presenting capacity, and downregulate the expression of co-stimulatory molecules-processes that facilitate immune evasion and persistent survival of pathogens in the host [25]. In contrast, IL-12 serves as a "bridge" connecting innate and adaptive immunity: it promotes the differentiation of naive CD4+ T cells into Th1 cells, which secrete IFN-γ to enhance the ability of macrophages to clear intracellular pathogens [26,27]. Further cytokine analysis in this study revealed key shifts in macrophage response. In BVDV and *B. abortus* A19 co-infected cells, we observed a marked upregulation of the immunosuppressive cytokine IL-10 at both mRNA and protein levels. Concurrently, the expression of key cytokines associated with a pro-inflammatory, Th1-polarized immune response (including IL-12, IFN-γ, and TNF-α) was significantly suppressed compared to infection with *B. abortus* A19 alone. These results indicate that BVDV infection induces increased IL-10 expression in RAW264.7 cells, thereby suppressing the inflammatory and immune responses triggered by RAW264.7 cells.

We therefore propose that BVDV infection drives macrophages toward an immunomodulatory state that is disadvantageous for bacterial clearance. The upregulated IL-10 may inhibit macrophage antimicrobial activity, while the dampened Th1-type cytokine response reduces immune activation. A response critical for activating macrophages to clear intracellular *Brucella* [28]. Collectively, this virus-induced reprogramming of the cytokine network appears to directly promote the intracellular survival and replication of *B. abortus* A19, providing a mechanistic explanation for the enhanced bacterial survival observed both in macrophages and in co-infected mice.

Our findings directly impact brucellosis management in field conditions. The observed immunosuppression suggests that BVDV endemicity may critically undermine

the efficacy of the *Brucella* vaccine, explaining suboptimal control in some vaccinated herds.

We therefore advocate for an integrated control strategy. Screening herds for BVDV before the *Brucella* vaccination should be considered to identify populations at risk of vaccine failure. Moreover, incorporating BVDV vaccination into brucellosis control programs in co-endemic areas could stabilize general herd immunity and potentially enhance the *Brucella* vaccine efficacy.

Based on the broader implications of our findings, we suggest that control programs targeting a single pathogen may have limited success in field settings where co-infections are prevalent. Our work provides a framework for studying similar interactions between immunomodulatory viruses and chronic bacterial pathogens, advocating for a shift toward more holistic, system-based approaches to animal disease management. Ultimately, such integrated strategies promise to improve animal welfare, livestock productivity, and public health outcomes for this major zoonosis.

In summary, this study confirms that BVDV infection can synergistically disrupt the immune protective effect of the *B. abortus* A19 vaccine through multiple mechanisms. These results provide a mechanistic explanation for the suboptimal performance of brucellosis vaccines in field settings and support the development of integrated control strategies that address BVDV co-infection. Adopting such a coordinated approach could enhance livestock health, improve productivity, and reduce the zoonotic risk of brucellosis. However, this study has certain limitations: its results need to be further validated in naturally infected livestock under field conditions to provide more optimal solutions for clinical practice.

Declarations

Availability of Data and Materials: The datasets generated and/or analyzed during the current study are available from the corresponding author/s (HZ, JG) upon reasonable request.

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Competing Interests: The authors declared that there is no conflict of interest.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the article, tables and figures were not written/created by AI and AI-assisted Technologies.

Author Contributions: Conceived of or designed study: H Z, J G; Performed research: YJ C; Analyzed data: YD Z, JH Z; Wrote the paper: YJ C.

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