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**Research article**

# The impact of helminth infections on the effectiveness of brucellosis immunoprophylaxis in cattle

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**Abstract**

Background and Aim. Brucellosis remains one of the most significant bacterial zoonoses affecting livestock productivity and public health worldwide. Vaccination is the cornerstone of bovine brucellosis control; however, its efficacy may be reduced by factors that affect the host immune response, including parasitic infections. Helminths are known to modulate immune and may suppress vaccine-induced antibody production. This study aimed to evaluate the effect of helminth infestation on antibody production following vaccination with Brucella abortus strain 82 and to assess the impact of prior anthelmintic treatment.

Materials and Methods. A total of 20 cattle were divided into two groups (n = 10 each). Helminth infections were confirmed in all animals using coproscopic and larvoscopic methods, including the Fulleborn flotation technique. The experimental group received anthelmintic treatment (Monizen forte, 1 µL/20 kg body weight, orally) before vaccination, whereas the control group was vaccinated without prior deworming. All animals were immunized with B. abortus strain 82 (5 µL, subcutaneously). Blood samples were collected 26 days after vaccination, and antibody responses were assessed using the indirect hemagglutination assay, agglutination test, and thecomplement fixation test.

Results. Parasitological analysis revealed mixed helminth infections *(Dictyocaulus* spp., strongylates, *Nematodirus* spp., and *Moniezia* spp.) in allanimals. Anthelmintic treatment demonstrated high efficacy, resulting in the complete elimination of strongylates and monieziosis. Serological results showed significantly stronger and more consistent antibody responses in the treated group compared with untreated controls, which exhibited lower titers and heterogeneous reactions.

Conclusion. Helminth infestation adversely affects post-vaccination humoral immunity against brucellosis in cattle. Pre-vaccination deworming enhances antibody production and may improve the effectiveness of brucellosis control programs.

**Keywords:** Brucellosis; *Brucella abortus;* helminth infestation; vaccination; anthelmintic treatment; cattle immunity.

**Introduction**

Brucellosis is one of the most widespread bacterial zoonosesaffecting both animals and humans worldwide. The disease is caused by Gram-negative bacteria of the genus Brucella, which are facultative intracellular pathogens capable of infecting a wide range of domestic and wild animal species. In cattle, the main etiological agent is *Brucella abortus*, which causes abortion, infertility, reduced milk production, and other reproductive disorders. These pathological effects lead to considerable economic losses in livestock production and pose a serious threat to food safety and public health [1–6].

Despite long-term control programs, involving vaccination, veterinary surveillance, and sanitary measures, brucellosis remains endemic in many regions of the world. In countries with well-developed livestock industries, the disease remains one of the major infectious constraints affecting cattle production and requires the continuous improvement of preventive strategies and vaccination approaches [7–9].

Vaccination remains the main method for the specific prevention of bovine brucellosis. Live attenuated vaccines, such as *Brucella abortus* strain S19 and RB51 are widely used in cattle to induce protective immunity and reduce the spread of infection within animal populations. These vaccines stimulate both humoral and cell-mediated immune responses, which together contribute to protection against infection. However, because *Brucella* spp. are facultative intracellular pathogens that replicate within macrophages, effective immunity largely depends on the activation of cellular immune mechanisms. In particular, T helper type 1 (Th1) cells play a critical role in protective immunity by producing interferon-gamma (IFN-γ), which activates macrophages and enhances their bactericidal activity against intracellular bacteria. Humoral immunity also contributes to host defense, particularly in the later stages of infection, when antibodies promote bacterial opsonization and facilitate phagocytosis [7, 8, 10–13].

However, the effectiveness of vaccination programs may vary considerably depending on several biological and environmental factors. The development of post-vaccination immunity may be influenced by the physiological status of animals, nutritional deficiencies, stress, management practices, and the presence of concurrent infections and other immunosuppressive factors [9, 14, 15]. Changes in the immunobiological reactivity of animals may therefore lead to reduced vaccine-induced immune responses and reduced effectiveness of preventive measures.

Parasitic infections are among the factors that can substantially influence the immune status of animals. Parasites have pronounced immunomodulatory properties and can significantly alter both innate and adaptive immune responses in the host. Numerous studies have demonstrated that chronic parasitic infections can lead to secondary immunodeficiency, reduce host resistance, and increase susceptibility to infectious diseases of various etiologies [14, 16].

Helminthiases comprise a large group of parasitic diseases caused by helminths, including nematodes, cestodes, and trematodes. These parasites are widely distributed among livestock worldwide. In cattle, the most common helminths include *Ostertagia, Haemonchus, Trichostrongylus, Fasciola,* and *Moniezia.* Helminths can persist in the host for long periods, causing chronic infections associated with metabolic disorders, decreased productivity, and modulation of the host immune system [16–18].

A characteristic feature of helminth infections is their ability to induce a T helper type 2 (Th2) immune response. This response is characterized by increased production of cytokines such as interleukin-4 (IL-4), IL-5, and IL-13, the activation of eosinophils and mast cells, and enhanced production of immunoglobulin E (IgE). The Th2-mediated immune response plays a crucial role in eliminating multicellular parasites from the host. However, this immune polarization may simultaneously suppress Th1-mediated immune responses that are required for effective protection against intracellular pathogens, including bacteria of the genus *Brucella* [16, 17, 19, 20].

In addition to inducing Th1/Th2 immune polarization, helminths can also activate regulatory mechanisms of the immune system. These include the expansion of regulatory T cells (Treg) and increased production of anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β). Such mechanisms reduce inflammatory responses and promote parasite survival, but may simultaneously suppress protective immune responses against unrelated pathogens or vaccines [18].

In recent years, increasing attention has been paid to the effects of helminth infections on vaccine-induced immunity. Experimental and epidemiological studies have demonstrated that chronic helminth infections can reduce the immunogenicity and protective efficacy of vaccines against various infectious diseases. These effects are mainly associated with parasite-induced immunomodulation and suppression of Th1-type immune responses required for effective vaccine-mediated protection [21, 22].

In livestock production systems, this interaction between parasitic infections and vaccination outcomes is of particular importance. Helminth infections are widespread in cattle populations and often occur as chronic subclinical infections that remain undetected during routine veterinary monitoring. Although such infections may not always cause obvious clinical signs, they can significantly alter the immunological reactivity of animals. Consequently, helminth infection in cattle may reduce the effectiveness of vaccination against infectious diseases, including brucellosis [6, 15].

A better understanding of the interactions between parasitic and bacterial infections may contribute to improving vaccination strategies, increasing the effectiveness of immunoprophylaxis, and optimizing disease control programs in livestock production.

## Materials and Methods

### *Ethical approval*

All procedures involving animals were carried out in accordance with generally accepted veterinary and ethical standards for the use of animals in scientific research.

*Animals and study design*

The study was conducted on 4-year-old red-steppe cows (n = 20) on a brucellosis-free farm in the Gunibsky District of the Republic of Dagestan. Due to limited material and technical resources, 20 animals were selected for the study. All animals were clinically examined before the experiment and serologically tested for brucellosis. Individual fecal samples were collected from each animal to determine the presence of helminth infections. According to the study design, the animals were divided into two groups of 10 animals each: an experimental group and a control group. Animals in the experimental group received anthelmintic treatment 7 days before vaccination., whereas animals in the control group were vaccinated without prior deworming.

*Parasitological examination*

Helminth infections were diagnosed using standard parasitological techniques, including coproscopic and larvoscopic examinations. Helminth eggs in fecal samples were detected using the Fulleborn flotation method. Parasitological analysis allowed the identification of helminths belonging to different taxonomic groups.

To evaluate the level of helminth infestation, the extent of the invasion (EI) was calculated as the percentage of infected animals in the examined population. After anthelmintic treatment, the extensive efficiency (EE) was determined to assess the therapeutic efficacy of the drug.

*Anthelmintic treatment*

Animals in the experimental group were treated with a broad-spectrum anthelmintic drug, Monizen forte. The drug was administered orally as a single dose at a dose of 1 μL per 20 kg of body weight according to the manufacturer's instructions. Treatment efficacy was evaluated based on the absence of helminth eggs in fecal samples collected after treatment.

*Vaccination*

Following the parasitological examination and anthelmintic treatment, animals in both groups were vaccinated against brucellosis using a vaccine prepared from the low-agglutinogenic Brucella abortus strain No. 82. The vaccine was administered subcutaneously at a dose of 5 μL into the posterior third of the neck according to the recommended vaccination protocol.

Serological testing

Blood samples were collected from the animals 26 days after vaccination to assess the formation of post-vaccination antibodies. Serum samples were obtained by centrifugation and used for serological testing. Three serological diagnostic methods were employed: indirect hemagglutination test (IHA), agglutination test (AT), and complement fixation test (CFT).

The agglutination test and the complement fixation test were performed according to the procedures described in the official "Guidelines for the Diagnosis of Animal Brucellosis." The indirect hemagglutination test was conducted according to the manufacturer's instructions.

*Statistical analysis*

The obtained data were analyzed using descriptive statistical methods. The prevalence of helminth infections and the efficacy of anthelmintic treatment were expressed as percentages. The serological responses of animals in the experimental and control groups were compared to evaluate the influence of helminth infestation on the development of the immune response following vaccination.

**Results**

The objective of the present study was to investigate antibody production following vaccination with the Brucella abortus strain 82 vaccine in cattle under conditions of helminth infestation.

For this purpose, two groups of 10 animals each were formed. Before the experiment, all animals were examined for the presence of helminth infections using standard parasitological diagnostic methods. The results of the examination showed that the prevalence of helminth infection among the studied animals was 100%. Parasitological analysis revealed the presence of several groups of helminths, including nematodes (*Dictyocaulus* spp., strongylates, *Nematodirus* spp.) and cestodes (*Moniezia* spp.).

Considering the highprevalence of mixed helminth infections in the studied animals, a broad-spectrum anthelmintic drug, Monizen forte, was selected for deworming. The drug was administered to animals of the experimental group according to the manufacturer's instructions at a dosage of 1 ml per 20 kg of body weight. The results of the treatment effectiveness are presented in Table 1.

Table 1. Efficacy of Monizen forte in the experimental group of animals

| Names of diseases | Number of animals freed from parasites after treatment (heads) | Drug dosage (mL/kg) | Route of administration | Extent of the invasion (EI, %) | Extensive efficiency (EE, %) |
|---|---|---|---|---|---|
| Nematode infections | | | | | |
| Dictyocaulosis | 7 | 1 mL/20 kg body weight | Single dose, individually, orally | 70.0 | 79.3 |
| Strongylid infection | 10 | 1 mL/20 kg body weight | Single dose, individually, orally | 100.0 | 100.0 |
| Nematodirosis | 8 | 1 mL/20 kg body weight | Single dose, individually, orally | | |
| Cestode infections | | | | | |
| Monieziosis | 10 | 1 mL/20 kg body weight | Single dose, individually, orally | 100.0 | 100.0 |

The data presented in Table 1 demonstrate the high therapeutic effectiveness of the *Monizen* forte preparation against the helminth infections detected in the animals. In cases of strongylate infections and monieziosis, complete elimination of helminths from the animals' bodies was observed, with both the extent of the invasion (EI) and the extensive efficiency (EE) reaching 100%. High effectiveness was also observed against Nematodirus infections, where 8 out of 10 animals were freed from the parasites. Slightly lower but still significant effectiveness was recorded in the treatment of dictyocaulosis, where 7 animals recovered following treatment, corresponding to an EI of 70.0% and an EE of 79.3%.

Overall, the results indicate that *Monizen* forte exhibits a pronounced anthelmintic effect against the most common gastrointestinal and pulmonary helminths of cattle. The elimination of helminth infections prior to vaccination is particularly important, as parasitic infestations can negatively affect the immune system and alter the formation of post-vaccination immunity.

After evaluating the effectiveness of the anthelmintic treatment, the animals were vaccinated against brucellosis using a vaccine based on Brucella abortus strain 82. Serological examination of blood sera

was performed 26 days after vaccination using three diagnostic methods: the indirect hemagglutination test (IHA), the agglutination test (AT), and the complement fixation test (CFT). The results obtained in the experimental group are presented in Table 2.

Table 2. Results of serological testing of animal blood sera following treatment with the anthelmintic *Monizen* forte

| Serological tests | | | |
|---|---|---|---|
| No | IHA | SAT, ME | CFT |
| 1 | 1:200 # | 100 # | 1:20 # |
| 2 | 1:200 # | 100 # | 1:20 +++ |
| 3 | 1:200 # | 100 ++ | 1:20 # |
| 4 | 1:200 +++ | 100 ++ | 1:20 ++ |
| 5 | 1:200 # | 100 ++ | 1:20 # |
| 6 | 1:200 # | 100 # | 1:20 +++ |
| 7 | 1:200 # | 100 # | 1:20 # |
| 8 | 1:200 # | - | 1:20 ++ |
| 9 | 1:200 # | 100 +++ | 1:20 # |
| 10 | 1:200 +++ | 100 ++ | 1:20 # |

*Note: IHA: 1:200 #, +++ high antibody titers*
*SAT, ME: 100 #, +++ high antibody titers*
*SAT, ME: 100 ++ low antibody titers*
*SAT, ME: - no antibody titers*
*CFT: 1:20 #, +++, ++ high antibody titers*

Analysis of the data presented in Table 2 shows that animals of the experimental group, which received anthelmintic treatment prior to vaccination, developed a pronounced humoral immune response to the vaccine. Most animals demonstrated positive serological reactions in at least one of the diagnostic tests. In several animals (Nos. 4, 6, and 10), strong positive reactions were detected simultaneously in two or three tests, indicating a sufficiently high level of antibody production following vaccination.

For example, animal No. 4 demonstrated strong positive reactions (+++) in the IHA and AT tests and a positive reaction (++) in the complement fixation test. Animal No. 6 showed strong antibody titers (+++) in both the IHA and CFT tests. Similarly, animal No. 10 exhibited strong positive reactions (+++) in the IHA test and moderate positive reactions (++) in the AT test. These results indicate active formation of post-vaccination antibodies and confirm the development of a specific immune response to the brucellosis vaccine.

At the same time, several animals showed weaker or borderline reactions in individual tests. However, the presence of positive reactions in different serological assays suggests that the vaccinated animals developed detectable levels of antibodies against Brucella antigens.

In contrast, the results obtained in the control group (Table 3), where animals were vaccinated without prior anthelmintic treatment, showed a less pronounced immune response. Although some animals demonstrated positive serological reactions, the overall antibody response was weaker and more inconsistent compared to the experimental group.

Table 3. Results of serological testing of animals in the control group (without anthelmintic treatment)

| Serological tests | | | |
|---|---|---|---|
| No | IHA | SAT, ME | CFT |
| 1 | 1:200 +++ | 100 ++ | - |
| 2 | 1:200 # | 100 + | 1:20 + |
| 3 | 1:200 +++ | 100 +++ | 1:20 + |
| 4 | 1:200 # | 100 # | 1:20 # |

Continuation of Table 3

| | | | |
|---|---|---|---|
| 5 | 1:200 +++ | 100 ++ | - |
| 6 | - | 100 ++++ | 1:20 + |
| 7 | - | - | 1:20 +++ |
| 8 | 1:200 +++ | - | - |
| 9 | 1:200 +++ | 100 ++ | 1:20 # |
| 10 | 1:200 +++ | - | - |

*Note: IHA: 1:200 #, +++ high antibody titers*
*IHA: - no antibody titers*
*SAT, ME: 100 #, +++ high antibody titers*
*SAT, ME: 100 ++ low antibody titers*
*SAT, ME: - no antibody titers*
*CFT: 1:20 #, +++ high antibody titers*
*CFT: 1:20 + low antibody titers*
*CFT: - no antibody titers*

In the control group, several animals demonstrated negative reactions in one or more serological tests. For example, animals Nos. 5, 7, 8, and 10 showed negative reactions in two diagnostic assays. In addition, antibody titers were generally lower, and strong positive reactions (+++) were observed less consistently across the different tests. The uneven distribution of serological responses suggests that helminth infections may have negatively influenced the development of post-vaccination immunity.

The comparison of the serological results between the experimental and control groups clearly demonstrates that animals receiving anthelmintic treatment prior to vaccination developed a more pronounced and consistent immune response. In the experimental group, positive reactions were detected more frequently and were characterized by higher antibody titers across multiple serological tests.

The observed differences in the immune response may be explained by the immunomodulatory effects of helminths. Helminth infections are known to induce a Th2-dominated immune response and activate regulatory immune mechanisms that suppress Th1-mediated immunity. Since protective immunity against Brucella relies largely on Th1-type immune responses, the presence of helminths may interfere with the development of effective post-vaccination immunity.

By eliminating helminths before vaccination, the immune system of animals in the experimental group was likely able to respond more efficiently to the vaccine antigen. This may have contributed to the more active production of antibodies and the stronger serological reactions observed in this group.

Thus, the results obtained in the present study demonstrate the importance of conducting deworming procedures prior to vaccination of cattle against brucellosis. The use of an effective anthelmintic drug allowed elimination of helminth infections and contributed to the development of a more pronounced post-vaccination immune response.

These findings confirm that helminth infections can negatively affect the immunological reactivity of animals and reduce the effectiveness of vaccination programs. Therefore, the integration of antiparasitic treatment into preventive veterinary programs may significantly improve the effectiveness of immunoprophylaxis against brucellosis in cattle.

**Discussion**

The results obtained in the present study clearly demonstrate that helminth infections may considerably influence the formation of immune responses following vaccination against brucellosis in cattle. Animals that underwent deworming before vaccination developed a more pronounced humoral immune response compared to animals that remained infected with helminths.

The observed differences between the experimental and control groups can be explained by the immunomodulatory properties of helminths. Numerous studies have shown that helminth infections are capable of altering the host immune system by inducing a Th2-dominated immune response and activating regulatory immune pathways. These mechanisms promote parasite survival but may

simultaneously suppress Th1-mediated immune responses, which are essential for protection against intracellular pathogens such as Brucella. Since effective immunity against brucellosis largely depends on the activation of cellular immune mechanisms and the production of interferon-gamma, helminth-induced immune modulation may interfere with the formation of protective post-vaccination immunity.

In the present study, animals treated with the anthelmintic drug Monizen forte demonstrated stronger and more consistent antibody responses following vaccination. The higher frequency of positive serological reactions and the presence of strong antibody titers in several diagnostic tests suggest that elimination of helminths prior to vaccination creates more favorable conditions for the development of immune responses to the vaccine antigen.

Conversely, animals in the control group, which remained infected with helminths, demonstrated weaker and more inconsistent serological reactions. The presence of negative reactions in several diagnostic assays and lower antibody titers indicate that helminth infections may reduce the immunogenicity of the vaccine. These findings are consistent with previously reported data indicating that parasitic infections may suppress immune responses to vaccines and reduce their effectiveness.

The results of this study therefore confirm the importance of considering parasitic infections as a significant factor influencing the outcome of vaccination programs in livestock. In regions where helminth infections are widespread, failure to eliminate parasites prior to vaccination may lead to inadequate development of post-vaccination immunity and consequently reduce the effectiveness of disease control measures.

The use of effective anthelmintic treatment prior to vaccination may therefore represent an important component of integrated veterinary preventive programs. Such an approach may improve the immunological responsiveness of animals and contribute to the formation of a stronger and more stable immune response following vaccination against brucellosis.

**Conclusion**

The results of the present study demonstrated that the administration of the anthelmintic drug Monizen forte did not produce any negative effects on the physiological condition of the animals during the experimental period. At the same time, the drug showed high anthelmintic efficacy against the helminth infections detected in the studied cattle, including nematodes and cestodes.

The obtained data indicate that helminth infestation may significantly influence the formation of the immune response following vaccination with the Brucella abortus strain 82 vaccine. Animals that received anthelmintic treatment prior to vaccination developed a more pronounced antibody response compared to the control group, which remained infected with helminths. This suggests that helminth infections can reduce the effectiveness of immunoprophylaxis by modulating the immune system and interfering with the development of vaccine-induced immunity.

Based on the results obtained, it is recommended to carry out deworming of cattle 10–14 days prior to vaccination against brucellosis. The implementation of this approach may contribute to the formation of a stronger and more stable immune response following vaccination and may therefore improve the overall effectiveness of brucellosis prevention programs in cattle.

**Authors' Contributions**

MM: concept development, visualization, and manuscript writing. ShG: formal analysis and resource provision. AKh: methodology development and research. EY: research and manuscript editing. PA: scientific supervision.

**Conflicts of Interes**

The authors declare that they have no conflicts of interest.

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