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HISTOPATHOLOGICAL EVALUATION OF LYMPH NODES IN CATTLE WITH TUBERCULOSIS: CORRELATION WITH INTRAVITAL DIAGNOSTIC METHODS (TUBERCULIN TEST AND PCR)

AVALIAÇÃO HISTOPATOLÓGICA DE LINFONODOS EM BOVINOS COM TUBERCULOSE: CORRELAÇÃO COM MÉTODOS DE DIAGNÓSTICO INTRAVITAL (TESTE DE TUBERCULINA E PCR)

EVALUACIÓN HISTOPATOLÓGICA DE LOS GANGLIOS LINFÁTICOS DE BOVINOS TUBERCULOSOS: CORRELACIÓN CON LOS MÉTODOS DE DIAGNÓSTICO INTRAVITAL (PRUEBA DE LA TUBERCULINA Y PCR)

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ABSTRACT

Objective: To evaluate the main diagnostic methods used in the detection of bovine tuberculosis, focusing on the combination of histopathological techniques, such as Hematoxylin-Eosin (H&E) and Ziehl-Neelsen (ZN), with the polymerase chain reaction (PCR), and to correlate them with the results of the intradermal tuberculin test. Bovine tuberculosis, caused by Mycobacterium bovis, is a zoonotic disease with a major economic and public health impact and is difficult to diagnose, especially in the early stages of infection. The tuberculin test is widely used, but has limitations in terms of specificity and sensitivity. Histopathological and molecular techniques, such as H&E, ZN and PCR, have shown efficacy in identifying granulomatous lesions and detecting the pathogen, even in more advanced stages of the disease. This literature review analyzed national and international studies on the application of these methodologies in the diagnosis of bovine tuberculosis, highlighting the clinical protocols, the effectiveness of the methods and the limitations associated with each technique. The results show that the combination of these diagnostic methods offers a more sensitive and accurate approach to the detection of M. bovis, with the need for further research into the standardization of protocols and validation at different stages of the disease.

Keywords: Diagnosis. Histopathology. Bovine tuberculosis. Tuberculin. PCR.

RESUMO

Objetivo: Avaliar os principais métodos diagnósticos utilizados na detecção de tuberculose bovina, com foco na combinação das técnicas histopatológicas, como Hematoxilina-Eosina (H&E) e Ziehl-Neelsen (ZN), com a reação em cadeia da polimerase (PCR), e correlacioná-los com os resultados do teste de tuberculina intradérmica. A tuberculose bovina, causada pelo Mycobacterium bovis, é uma doença zoonótica de grande impacto econômico e de saúde pública, sendo difícil de diagnosticar, especialmente nos estágios iniciais da infecção. O teste de tuberculina é amplamente utilizado, mas apresenta limitações quanto à especificidade e sensibilidade. As técnicas histopatológicas e moleculares, como H&E, ZN e PCR, têm mostrado eficácia na identificação de lesões granulomatosas e na detecção do agente patogênico, mesmo em estágios mais avançados da doença. Esta revisão bibliográfica analisou estudos nacionais e internacionais sobre a aplicação dessas metodologias no diagnóstico da tuberculose bovina, destacando os protocolos clínicos, a eficácia dos métodos e as limitações associadas a cada técnica. Os resultados demonstram que a

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combinação desses métodos diagnósticos oferece uma abordagem mais sensível e precisa para a detecção de M. bovis, com a necessidade de mais pesquisas para a padronização de protocolos e validação em diferentes estágios da doença.

Palavras-chave: Diagnóstico. Histopatologia. Tuberculose bovina. Tuberculina. PCR.

RESUMEN
Objetivo: Evaluar los principales métodos de diagnóstico utilizados en la detección de la tuberculosis bovina, centrándose en la combinación de técnicas histopatológicas, como la hematoxilina-eosina (H&E) y Ziehl-Neelsen (ZN), con la reacción en cadena de la polimerasa (PCR), y correlacionarlas con los resultados de la intradermotuberculinización. La tuberculosis bovina, causada por Mycobacterium bovis, es una enfermedad zoonótica de gran repercusión económica y para la salud pública y difícil de diagnosticar, sobre todo en las primeras fases de la infección. La prueba de la tuberculina se utiliza ampliamente, pero tiene limitaciones en cuanto a especificidad y sensibilidad. Las técnicas histopatológicas y moleculares, como H&E, ZN y PCR, han resultado eficaces para identificar lesiones granulomatosas y detectar el patógeno, incluso en estadios más avanzados de la enfermedad. Esta revisión bibliográfica analizó estudios nacionales e internacionales sobre la aplicación de estas metodologías en el diagnóstico de la tuberculosis bovina, destacando los protocolos clínicos, la eficacia de los métodos y las limitaciones asociadas a cada técnica. Los resultados muestran que la combinación de estos métodos de diagnóstico ofrece un enfoque más sensible y preciso para la detección de M. bovis, con la necesidad de seguir investigando para estandarizar los protocolos y validarlos en diferentes etapas de la enfermedad.

Palabras clave: Diagnóstico. Histopatología. Tuberculosis bovina. Tuberculina. PCR.

INTRODUCTION

Bovine tuberculosis (TB), caused by *Mycobacterium bovis*, is a zoonosis of global relevance, associated with serious impacts on public health and the livestock economy, especially in countries with extensive production systems (Waters *et al*., 2020). In addition to posing a risk to human health, the presence of the disease in the herd imposes sanitary and commercial barriers, directly affecting competitiveness in the international market (Meikle *et al*., 2021).

The control of bovine tuberculosis depends fundamentally on the early identification of infected animals. However, detection of the disease is challenging, especially in subclinical infections, in which the immune response may be insufficient to generate positive results in conventional tests (Barandiaran *et al*., 2022). The widely used official diagnostic method is the intradermal tuberculin test, which, although it has high specificity, has variable sensitivity, being influenced by factors such as the stage of infection, the immune status of the animals, and previous exposure to environmental mycobacteria (Álvarez *et al*., 2021; De la Rua-Domenech *et al*., 2006).

In this context, the use of complementary methods becomes indispensable. Histopathological evaluation of affected lymph nodes and organs allows the identification of granulomatous lesions characteristic of M. bovis infection, with central caseous necrosis, epithelioid cells, multinucleated giant cells of the Langhans type, and lymphoplasmacytic infiltrate (Carpenter *et al*., 2021). The Ziehl-Neelsen technique, used for the detection of acid-fast bacilli (AFB), has good specificity, but its sensitivity is limited in paucibacillary lesions, which are common in the early stages of the disease (Meikle *et al*., 2021).

At the same time, advances in molecular methodologies, especially polymerase chain reaction (PCR), have provided significant gains in diagnostic accuracy, allowing the detection of M. bovis DNA directly in tissue samples, even in the absence of bacilli detectable by Ziehl-Neelsen staining (Barandiaran *et al*., 2022). However, factors such as sample quality, the presence of inhibitors, and bacterial load can still interfere with PCR performance.

Therefore, the correlation between histopathological findings and the results of intravital diagnostic methods, such as the tuberculin intradermal test and PCR, is essential to improve the detection strategies of bovine tuberculosis, especially in cases with a reduced bacterial load. Thus, this study aims to evaluate the efficacy of the association between histopathological methods (Hematoxylin and Eosin and Ziehl-

Neelsen) and PCR in the detection of Mycobacterium bovis in bovine lymph nodes with suspected tuberculosis, correlating these findings with the results of the intradermal tuberculin test.

METHODOLOGY

In order to carry out this study, a systematic literature review of scientific articles, dissertations, and relevant publications on the diagnosis of bovine tuberculosis and the methods used for its detection was conducted, with emphasis on the combination of histopathological, PCR, and Ziehl-Neelsen techniques. The search for sources was carried out in the scientific databases PubMed, Google Scholar and Scopus, focusing on publications from the last 20 years, in order to gather the most recent studies on the subject. The inclusion criteria were: clinical articles, case studies, systematic reviews, and clinical trials discussing the application of different diagnostic methods in the detection of *Mycobacterium bovis* in cattle with tuberculosis.

The collected data were analyzed to identify the main findings on the efficacy of tuberculin tests, histopathology, Ziehl-Neelsen staining, and polymerase chain reaction (PCR) in the diagnosis of the disease. The review focused on the protocols used, the post-treatment results, the sensitivity and specificity of the methods, and the limitations of each approach. The studies were evaluated based on the methodological quality and relevance of the results presented, thus seeking to provide a comprehensive view of the current diagnostic methods for bovine tuberculosis.

RESULTS AND DISCUSSION

Bovine tuberculosis, caused by *Mycobacterium bovis*, is one of the most important zoonoses in the context of livestock, significantly impacting public health and the economy. Effective control of bovine tuberculosis depends on the early identification of infected animals. However, diagnosis represents a challenge, especially in cattle with subclinical infections or in the early stages, in which there is no evident clinical manifestation and the bacterial load may be insufficient for detection by some conventional methods (Álvarez *et al*., 2021). Over the years, several diagnostic methods have been developed, including tuberculinization, postmortem inspection, histopathological tests, such as H&E and ZN staining, and polymerase chain reaction (PCR), which has stood out as an additional high-sensitivity tool (Waters *et al.,* 2011).

HISTOPATHOLOGY IN THE DETECTION OF BOVINE TUBERCULOSIS

Histopathological examination has been shown to be an effective tool for the diagnosis of tuberculosis, since it allows the visualization of the granulomatous lesions typical of the disease. These lesions, formed by caseous necrosis, epithelial cells, lymphocytes, plasma cells, and Langhans giants, represent the characteristics of the chronic inflammatory response triggered by *M. bovis infection* (França *et al.,* 2016; Furlanetto *et al.,* 2012).

According to Varello *et al.* (2008), histopathology has a sensitivity of up to 93% and specificity of more than 83%, being extremely effective in the detection of lesions compatible with *M. bovis* infection. Hematoxylin and Eosin (H&E) staining allows the identification of the evolutionary stages of granulomas, from the initial phases, with lymphoplasmacytic inflammatory infiltrate and macrophages, to more advanced lesions with necrosis and mineralization. This offers a detailed view of the evolution of the host's immune response to infection (Carpenter *et al.,* 2021; Meikle *et al.,* 2021). In addition, several studies highlight the high sensitivity of this technique, capable of detecting lesions even in chronic phases of the disease, even in the absence of other clinical or laboratory symptoms (Varello *et al.,* 2008).

TUBERCULINATION IN THE DIAGNOSIS OF BOVINE TUBERCULOSIS

The tuberculination technique is the most common technique to identify cattle infected with Mycobacterium bovis, the etiological agent of bovine tuberculosis. This test is crucial for the management of the disease, particularly in national tuberculosis elimination programs in cattle, and is widely used in several nations, including Brazil (França *et al.,* 2016; Ávila *et al.,* 2012). Tuberculosis in cattle results in huge economic losses due to the deterioration of carcasses and the decrease in meat and milk production, in addition to being a zoonosis that poses a danger to public health (Michel *et al.,* 2010).

Tuberculination is based on the application of an antigen from *Mycobacterium bovis*, PPD (purified protein derivative), directly on the animal's skin. Usually, the application is performed in the cervical region or on the eyelid. After receiving the PPD, the animal is followed for 72 hours to confirm the existence of a late hypersensitivity reaction. When an animal has already been exposed to or infected by *M. bovis,* the immune system reacts to the substance with the formation of an edema or nodule,

signaling a positive result for tuberculosis. This reaction is assessed by the amount of swelling or nodule that forms in the area of application (Monaghan *et al.,* 1994).

Despite being a practical and affordable method, tuberculinization has variable sensitivity, between 68% and 95%, and specificity that can reach 98%, depending on the animal's immunological conditions, the prevalence of environmental mycobacteria in the region, and the technique used (Álvarez *et al*., 2021; De la Rua-Domenech *et al*., 2006). The main concern is the risk of false-positive reactions, such as in animals exposed to other mycobacterial species, such as *Mycobacterium avium*, or in vaccines that employ strains of *Mycobacterium bovis* to immunize animals against tuberculosis. This makes it difficult to understand the test, especially in regions with a high incidence of other mycobacteria or where vaccines are frequently administered. In addition, the test may not identify initial or subclinical infections, since the immune response may not be sufficient to elicit a noticeable response, leading to false-negatives, especially in newly infected animals or those with a reduced bacterial load (França *et al.,* 2016; Furlanetto *et al.,* 2012).

Due to these limitations, it is suggested that additional methods be applied to confirm the diagnosis of tuberculosis in cattle. Histopathological analysis and Ziehl-Neelsen staining are commonly used in conjunction with tuberculin testing in postmortem inspection. For example, histopathology makes it possible to identify the granulomatous lesions typical of the disease, while PCR is able to identify the DNA of *M. bovis* in compromised tissues, even in the early stages of infection. The combination of these procedures improves the accuracy of the diagnosis, particularly when the tuberculin test is not adequate to provide definitive results (Varello et al., 2008; Furlanetto et al., 2012).

ZIEHL-NEELSEN STAINING

The ZN technique is often employed to identify acid-fast bacilli (AFB), a traditional indicator of *Mycobacterium bovis* infection. Despite being a simple and low-cost procedure, ZN has considerable restrictions, especially when used in histological sections of lesions already in more advanced stages. The existence of AFB can be limited, which often leads to a lower-than-expected detection rate (Furlanetto *et al*., 2012; Palmer & Waters, 2006).

The reduced sensitivity of the method can be explained by the loss of bacterial structure in granulomas, caused by the host's immunological reactions that involve the bacteria in regions of necrosis or calcification (Andrade *et al.,* 1991). Therefore, integration with other techniques, such as histopathology, is crucial to ensure greater accuracy in diagnosis.

Even with its limitations, ZN persists as a relevant auxiliary method, especially when the results are positive, since it confirms the existence of M. bovis in the analyzed material. The joint use of ZN and other diagnostic methods is suggested to improve the accuracy of the results (Varello *et al.,* 2008).

PCR TECHNIQUE

The polymerase chain reaction technique has been shown to be extremely sensitive for the molecular diagnosis of tuberculosis in cattle. PCR makes it possible to identify *M. bovis* DNA in granulomatous lesions, even when the number of bacilli in the samples is extremely low, which can happen in the early stages of the disease (Waters *et al.,* 2011). Recent research has proven the effectiveness of PCR in detecting *M. bovis* in samples of lymph nodes, liver, lung, and other tissues from cattle with suspected tuberculosis, even when ZN staining does not identify the bacilli (Furlanetto *et al.,* 2012) Therefore, this method provides a promising option for situations where conventional methods are unable to deliver conclusive results.

PCR has also been shown to be useful for detecting M. bovis DNA in samples with reduced bacterial load, which is especially relevant in the diagnosis of subclinical infections or in early stages of the disease, where the immune response is not yet strong enough to generate antibodies detectable in serological tests (Waters *et al.,* 2011). The combination of PCR and histopathological techniques considerably improves the accuracy of the diagnosis, as both complement each other by identifying various aspects of the infection.

COMPARISON BETWEEN DIAGNOSTIC METHODS

Despite the widespread use of intradermal tuberculinization, it does not guarantee 100% reliability, especially in animals in the early stages of infection or when there are cross-interactions with other mycobacteria (Monaghan *et al.,* 1994). In this scenario, the use of additional techniques, such as histopathology, Ziehl-Neelsen and

PCR, has demonstrated a great capacity to improve the accuracy of the diagnosis. The combination of these techniques provides a more solid strategy, capable of identifying positive cases that could otherwise be neglected by the exclusive use of a single diagnostic method (Medeiros *et al.,* 2012).

FINAL CONSIDERATIONS

The review of studies on the diagnosis of bovine tuberculosis highlights the importance of combining histopathological methods, such as H&E and Ziehl-Neelsen staining, with molecular techniques such as PCR. While tuberculinization and macroscopic inspection are valuable tools, complementary methods offer an effective solution for diagnosing disease at different stages of infection. The combined use of these methods not only increases the sensitivity of the diagnosis, but also contributes to a better understanding of the epidemiology of the disease and the development of more effective strategies for the control and eradication of tuberculosis in cattle herds.

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