Role of Acute Phase Protein in Infection of *Mycoplasma Bovis* in Small Ruminants

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Abstract

Mycoplasma Bovis (*M. bovis*) is an important pathogen of small ruminants, responsible for respiratory diseases, mastitis, and arthritis diseases that cause major losses in livestock. They have revealed acute-phase proteins (APPs) such as haptoglobin (Hp) and serum amyloid A (SAA) that describe the biomarkers for *M. bovis* infections in the acute phase. These proteins are synthesized during inflammatory cytokines and give an understanding of Systemic Inflammation and timely interventional measures both in clinical and sub-clinical conditions. Although other diagnostic methods, such as culture and PCR, are accurate but cumbersome and require a lot of resources, the APP-based diagnostic methods are quick, non-invasive, and ideal for field use. However, APP responses are nonspecific because they are elevated in many inflammatory disorders, and thus need to be used in conjunction with other diagnostics to enhance accuracy and specificity. Some limitations include the absence of reference standards for cut-offs and inter-laboratory variability in an assay that limits their overall use. However, there is a significant point of view for APPs not only in the diagnostic field but also in the assessment of the effectiveness of therapy, as well as the response to a vaccine, and the study of the progress of the disease. The accumulation of multiplex diagnostic platforms and real-time field-applicable APP testing might be a breakthrough in the management of *M. bovis* infections in small ruminants, which will fall under precision veterinary medicine. This chapter focuses on the function of APPs to become diagnostic and prognostic tools and examines their advantages, disadvantages, future trends, and trends, particularly in improving the overall health of livestock.

Keywords: *Mycoplasma bovis*, Acute Phase Proteins (APPs), Biomarkers, Small ruminants, Disease management

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Introduction

Mycoplasma Bovis (*M. bovis*) is an economically significant and highly adaptable pathogen of ruminant species, including cattle, sheep, and goats. As a member of the Mollicutes class, *M. bovis* lacks a cell wall and is naturally resistant to beta-lactam antibiotics, thereby complicating treatment strategies. *M. bovis* has been recognized globally as a major cause of respiratory diseases, mastitis, arthritis, and otitis in young calves and small ruminants since the 1960s. As the pathogen can cause both acute and chronic infections, it contributes to great economic losses in livestock production. However, these infections commonly cause losses in cow production, weight loss in cows, and increased treatment costs (Dudek et al., 2020).

M. bovis is a multifactorial pathogen, the pathogenicity is influenced by external stressors including overcrowding, climatic changes, transport, and poor farm management practices. Infection primarily involves the respiratory tract and causes bronchopneumonia, but it disseminates systemically to cause polyarthritis, mastitis, and otitis. Clinical signs of infection in small ruminants are commonly characterized as nasal discharge, coughing, dyspnea, elevated body temperatures, and joint inflammation. The disease can also be caused by subclinical infections that are hard to detect and facilitate disease spread. Now these subclinical cases are particularly concerning because animals can very often be healthy, allowing *M. bovis* to proliferate secretly in herds (Zhou et al., 2023).

M. bovis is transmitted by direct contact with infected and noninfected animals, respiratory aerosols, and consumption of contaminated milk. Vertical transmission by infected milk is common in dairy herds, particularly among nursing young, and horizontal transmission through contact or shared feeding and watering systems. Most infections occur in young or immunocompromised animals, and poor hygiene, lack of adequate biosecurity measures, and environmental factors, such as overcrowding and venting, contribute to infection rates. Stress or other concurrent infections immunosuppress *M. bovis,* causing it to become more susceptible in intensive farming systems, small ruminants are especially susceptible (Devi et al., 2021).

M. bovis infections are one of the most challenging diseases to diagnose. Early detection is difficult because the disease is absent of distinct clinical signs in the early stage. However, traditional diagnostic methods such as culture and the polymerase chain reaction (PCR) are-time-consuming resource resource-intensive, and require skilled personnel and specialized facilities, which are often unavailable in resource-limited settings. This often leads to diagnosis being late, and infections advancing and spreading. Recently, APPs, e.g., Hp and SAA, are reliable markers of early *M. bovis* infection. The expression of these proteins is induced in response to inflammation and infection, making them particular candidates for identifying subclinical and clinical cases of disease before the onset of severe disease (Dudek et al., 2020).

M. bovis infections in small ruminants have profound economic and health implications. Reduced feed efficiency, weight loss, and reduced productivity in infected animals have a pronounced impact on livestock producers. *M. bovis*-associated mastitis in dairy animals, besides causing milk yield and quality losses, also leads to significant economic losses. In addition, chronic respiratory infections can result in permanent lung damage, reduced performance, and decreased survival rates. The losses in the livestock industry from *M. bovis* are substantial, especially for dairy and beef herds, where *M. bovis* contributes to calf mortality, reduced carcass value, and increased treatment costs. As illustrated in Figure 1, economic losses are primarily attributed to mastitis (40%), respiratory diseases (30%), reduced weight gain and carcass value (20%), and treatment costs (10%) (Survey, 2023-2024). Losses due to *M. bovis* respiratory diseases alone amount to millions of dollars annually, emphasizing the urgent need for effective disease management strategies (Deeney et al., 2021).

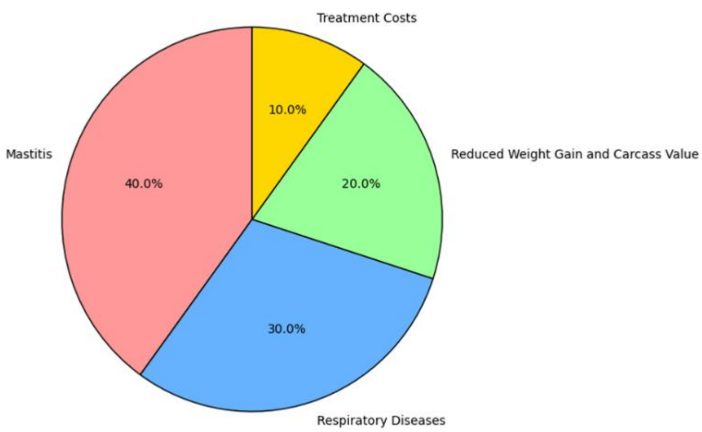

Fig. 1: Economic Losses Attributed to *Mycoplasma bovis* Infections

Pathophysiology of *M. bovis* Infection in Small Ruminants

Mechanism of Infection

Infection with *Mycoplasma Bovis* in small ruminants is not as simple as it has multiple ways by which this bacterium invades and persists in the host cell. Regarding pathogenicity, a pathogen can use strategies such as adhering to the host tissues, avoiding the host's immune response, and altering the host's cellular signaling pathways. Clathrin-dependent endocytosis (CDE) and lipid raft-mediated pathways are the major mechanisms by which *M. bovis* enters non-phagocytic cells (nPCs), including embryonic bovine lung cells. This process enables the bacteria to be released from the phagosome after being internalized (Li et al., 2024). The lipoprotein LppB is mandatory for adhesion since it binds to extracellular matrix components and enables endocytosis (Lan et al., 2023). Molecular chaperone host annexin A2 also increases during the infection and promotes both the adhesion and invasion of *M. bovis,* along with the regulation of inflammation (Zhang et al., 2022). *M. bovis* forms chronic infections by being protected phobic and therefore difficult to treat and manage. The secreted protein MbovP280 causes apoptosis in macrophages, which enhances immune evasion and persistent infection (Hoelzle et al., 2020). Despite complex approaches to invasion, the creation of preventions and treatments for *M. bovis* remains complex due to its ability to linger and avoid the host's immune system. It is at this point that these mechanisms can be understood to enhance good management practices of livestock.

Clinical Manifestations

Mycoplasma Bovis in small ruminants is characterized by various clinical signs that may affect the animal's health and productivity. The clinical signs depend on the involved Mycoplasma species, the host, and the health status of the animals. Their recognition is essential to diagnosis and treatment since these manifestations are part of a complex and varied clinical picture. *Mycoplasma Bovis* is known to cause bronchopneumonia, and its symptoms include coughing, nasal discharge, and dyspnoea. Infection leads to mastitis, which is inflammation of the mammary glands, which may even cause a decreased milk yield. The Mycoplasma infections can also present as arthritis, which results in lameness and joint swellings. Another sign is that keratoconjunctivitis usually implies eye inflammation and discharge (Dudek et al., 2020). It is also important to note that in addition to *Mycoplasma Bovis,* which is already a major concern, the other Mycoplasma species mentioned can also produce similar clinical signs in small ruminants, thereby further complicating the diagnosis and control of the disease.

Host Immune Response

The immune response of the host to *M. bovis* in small ruminants involves several mechanisms resulting in chronicity due to immune evasion by the pathogen. It can also suppress the functions of neutrophils and macrophages to swallow phagocytosis of the pathogen, increase the apoptosis of neutrophils and lymphocytes, and at the same time delay the apoptosis of macrophages. Immune dysfunction plays an important role (Askar et al., 2021). Also, it favors a T-helper 2 immune response, which provides limited help in the fight against the disease (Maunsell & Chase, 2019). That is why the overall cytokine response during the COVID-19 infection is characterized as inadequate, the synthesis of interferon-gamma (IFN-γ), which is one of the main cytokines for macrophage activation, in infected patients is uncertain, which also confirms the heterogeneity of the immune response (Maggioli et al., 2016). *M. bovis* infections are long-standing, which makes control a challenge, partly because of the weak immunity that results from routine vaccinations; however, recent approaches have shown that certain targeted vaccinations can boost immunity, although not enough to prevent infection completely. Knowledge of these immune events is fundamental in designing vaccines and other strategies to control and treat *M. bovis* infections in small ruminants (Arteche-Villasol et al., 2022).

Challenges in Detecting

Identification of Mycobacterium bovis in small ruminants thus remains daunting because of the weak diagnostic techniques and the generic nature of the disease. Molecular diagnostic tests, as earlier expounded, carry an element of specificity challenge, given general broad gene targets that may cause misdiagnosis (Mabe et al., 2024). Conventional methods of culture, which are assumed to be the 'gold standard' for diagnosis, take several weeks, and the technique can seldom identify infections caused by low bacterial load. Sample collection caused a further challenge to detection and confirmation because paucibacillary samples from small ruminants often produce low levels of *M. bovis,* and cross-reactions with non-tuberculous mycobacteria from shared pastures complicate the diagnostic tests (Deborah M. Cooke et al., 2024). LAMP and PCR offer a high degree of sensitivity and specificity but are likely to be problematic when applied in field conditions. Although molecular diagnostics and better knowledge of *M. bovis* transmission characteristics provide a basis for higher detection rates, the great reliance on conventional methods and the need for more specific assays remain challenging (Okella et al., 2023).

APPs in Infection and Inflammation

APPs play a key role in the host immune response to infection or inflammation, acting as early biomarkers for diagnosis and disease monitoring. The most commonly discussed APPs are plasma proteins that are primarily produced in the liver after the proinflammatory cytokines IL-1, IL-6, and TNF-α are released, using the acute phase reaction (Hasankhani et al., 2022). APP production is a characteristic of systemic inflammation directed towards neutralizing pathogens, activation of the complement system, and regulation of the immune response. Among the most studied key APPs in ruminants, haptoglobin, serum amyloid A, and C-reactive protein are the most studied (Stanke et al., 2023). In a similar developmental pattern, these proteins are induced under inflammatory conditions and have separate critical roles in immune and disease severity modulation. Just as important as anything that can simply inform us about infection, APPs not only indicate infection, but they also play a critical role in modulating the host immune response.

For example, opsonization and activation of complement, and regulation of tissue repair by inflammatory immune cells. In addition, APP levels correlate with the degree of infection and suggest associations with disease course and outcome. APPs are valuable diagnostic tools because they respond rapidly and specifically to inflammatory stimuli. As a marker of systemic inflammation, serum Hp and SAA levels in established *M. bovis* infections were found to be elevated in naturally infected *M. bovis* calves and may help to distinguish infected from healthy animals. From a diagnostic perspective APPs have many advantages. In veterinary settings, where early detection is crucial to effective disease management, they are sensitive indicators of early-stage infection and inflammation. Their diagnostic potential is especially illustrated by the large increases in SAA and Hp levels observed in infected calves (Güleç et al., 2022). In addition, APPs measurement is less invasive and quicker than traditional techniques, leading to quicker intervention and better animal health outcomes.

Role of APPs in *M. bovis* Infections in Small Ruminants

APPs are plasma proteins whose concentration dynamics are distinct in the conditions connected with infection, inflammation, or injury. These proteins are further described as positive APPs, which rise in the early acute phase response, or negative APPs, which fall. In ruminants, certain APPs are haptoglobin, serum amyloid A, and C-reactive proteins, and all of them have specific functions in the immune system response (Jain et al., 2011). APPs are mainly synthesized in hepatocytes in the liver in response to the action of the above pro-inflammatory cytokines including interleukin 1(IL-1), interleukin 6(IL-6) and tumor necrosis factor-alpha (TNF-α). Some of these cytokines are produced at the site of an inflammation caused by bacterial or viral pathogens or an injury, and they play significant roles in the control of APP synthesis (Kabu et al., 2023). APPs have several roles at the time of infection: manipulating the host immune response by depriving nutritive value matter from pathogens, aiding in the swallowing of microbes, and helping in tissue remodeling. Furthermore, their concentrations are proportional to viral load, which is important for diagnosing and evaluating inflammation. Diagnostic and prognostic applications of APPs are to serve as activation markers that signal a pathological process of infection or inflammation, and which provide an easy-to-identify abnormal state in animals. Unlike more specific diagnostic tests, APP measurements give an overall picture of the immune status of the host, which is very useful for early identification of subclinical infection and disease progression in veterinary. Since APPs are highly sensitive and quick to react with little complicated assays for their detection, they are now widely accepted in enabling effective animal health management and disease control interventions (Kushner & Mackiewicz, 2020).

APPs are useful in assessing immune response given *M. bovis* infections in small ruminants and defining the severity of the response. When infected with *M. bovis*, some species of APPs change significantly, such as Hp, SAA, and fibrinogen, indicating inflammation and the development of the disease (Çenesiz et al., 2023).

Specific APPs Elevated in *M. bovis* Infections

Haptoglobin (Hp)

HP is one of the most sensitive APPs during *M. bovis* infections and has been used as an indicator of inflammation, as well as intensity of infection. Its functions include sequestering free hemoglobin, thereby preventing toxic oxidative tissue damage and modulating immune response. Other research has indicated that Hp levels are substantially higher in *M. bovis* respiratory disease affected calves, corresponding to an increase from baseline blood Hp levels of 123.98 µg/ml of blood in apparently healthy calves to over 617.66 µg/ml for clinical cases of *M. bovis* respiratory diseases. This a marked increase underlines the role of Hp as a diagnostic criterion for systemic inflammatory disorder (Sameed Saher et al., 2024).

Serum Amyloid A (SAA)

SAA provides profound importance as an acute phase protein in *M. bovis* infections in cattle. Their levels rise considerably in response to inflammatory stimuli, and thus may serve as diagnostic markers for infections, as well as prognostic markers for chronic diseases. SAA is another APP that is known to increase during the acute phase of *M. bovis* infection and also enters the list of twenty. SAA also plays in the host's Immune defense by transporting immune cells to sites of infection and regulating inflammation. It also covers nutrient supply to the pathogen, and one of the most important nutrients highlighted is retinol. At the same time, SAA level differ significantly in infected and healthy calves: 30,12 and 3,98 µg/ml respectively, which could be crucial for *M. bovis* early diagnostics and monitoring (Sameed Saher et al., 2024).

Fibrinogen

Fibrinogen is the specific APP that is produced by the liver and is central to the manifestation of immune response to infections such as *Mycobacterium bovis* in cattle. Scientists have described higher concentrations of fibrinogen in cattle suffering from tuberculosis than in healthy animals, making it valuable for diagnosis. More APPs, including haptoglobin and serum amyloid A, also rise during *M. bovis* infection, indicating inflammation (Çenesiz et al., 2023). This increase in fibrinogen forms part of a general APP response that affords a complete APP profile, hence the suitability in the early diagnosis and monitoring of infections in animals. Its ability to increase in response to inflammatory stimuli makes it useful in veterinary diagnostic medicine; however, definitive diagnosis benefits from consideration of APPs collectively and in conjunction with other APPs as an index to provide insight into the acute phase response (Razavi et al., 2023).

Ceruloplasmin (Cp)

Cp is an APP with an important function associated with the immune response of cattle infected with *Mycobacterium bovis*. It has been shown that during infection Cp, along with other APPs, is produced at increased levels as part of the acute phase response, and appropriately related to the inflammatory state of the animal. The level of Cp in cattle with *M. bovis* infection increased by approximately 6.5 times in the same studies, pointing to Cp as a biomarker of inflammation and infection. It is also known that, like Cp, the other APPs, haptoglobin and serum amyloid A, are increased during infection adding favor to the acute phase response. Monitoring the elevation of Cp and other APPs can be used as a diagnostic tool for *M. bovis* infection in cattle to prevent an early spread. It is greatly useful in clinical assessment as the Cp level between the infected and healthy cattle are very different (Paksoy et al., 2024). Cp is a good marker of an acute phase response, but one must note that its levels can be influenced if the patient suffers from a viral infection or is stressed out, and this can make it hard to interpret results in a clinical setting.

Ferritin

In Cattle, infected with *Mycobacterium bovis*, ferritin is a key APP and an indicator of the inside inflammation due to tuberculosis (TB). Ferritin levels increase during the acute phase response, an important event for iron sequestration and immune modulation, according to research. Given that elevated ferritin levels are a feature of *M. bovis* infection, this response is particularly relevant in that respect (Çenesiz et al., 2023). Hepatocytes and macrophages are ferritin-producing cells, whose levels increase in inflammation, as a positive acute phase reactant. Severe cases of TB in unselected cattle were also shown to correlate with significant increases in APPs, particularly ferritin. Given that elevated ferritin levels are found in cattle with *M. bovis* diseases, they may also be biomarkers for diagnosing inflammatory diseases in cows and bulls (Değirmençay et al., 2022). Ferritin, along with other iron-related parameters, is a good marker of inflammatory conditions in calves with respiratory diseases. Ferritin levels may be good to monitor to understand how the immune response and treat TB in cattle, possibly leading to a means to treat TB. Ferritin is an important immune pathogenic factor because it has a dual role as an iron storage protein and an inflammatory mediator in diseases such as TB (Ali et al., 2023). However, while ferritin is a useful marker of inflammation, its elevation is not specific for *M. bovis* infection and can occur in other inflammatory conditions making it a less than specific diagnostic tool. Table 1 presents the summarize diagnostic features of the APPs.

Table 1: APPs and Their Diagnostic Features

APP Name	Function	Healthy Levels	Infected Levels	Diagnostic Relevance	References
Haptoglobin	Sequesters free hemoglobin	123.98 µg/ml	617.66 µg/ml	Early marker for inflammation	(Sameed Saher et al., 2024)
Serum Amyloid A	Transports immune cells, regulates inflammation	3.98 µg/ml	30.12 µg/ml	Monitors chronic infection	(Sameed Saher et al., 2024)
Fibrinogen	Central to clot formation and tissue repair	2-4 g/L	Elevated	Indicator of systemic inflammation	(Razavi et al., 2023)
Cp	Oxidative stress response, copper transport	30-60 mg/dL	Elevated	Associated with oxidative damage and infection	(Paksoy et al., 2024)
Ferritin	Regulates iron storage and immune modulation	50-150 µg/L	Elevated	Marker of immune activation and iron metabolism	(Ali et al., 2023)

Correlation between APP Levels and *M. bovis* Infection

The kinetics of APPs during *M. bovis* infections show significant differences in the acute phase as compared with chronic infections. In the beginning, APP including SAA and Hp increase swiftly based on pro-inflammatory cytokines like IL-1, IL-6, AND TNF- α which belong to innate immunity. This acute response is perhaps evident in such slight clinical symptoms as nasal discharge, arthritis, and dyspnea (Hasankhani et al., 2022). However, and more importantly, during chronic infections APP courses may stabilize or drop, which could be attributed to immune system weariness or immunosuppression. In addition, the comparison of APP levels between symptomatic and asymptomatic animals yields diagnostic values. While asymptomatic carriers may possess normal APPs, symptomatic animals show very high values thus making the APPs especially useful in differentiating clinical stages of *M. bovis* infections (Sameed Saher et al., 2024).

Impact of APPs on Disease Progression

Abnormality in the APPs during *M. bovis* infection is a sign of peripheral inflammation, which leads to tissue injury and worsening of the disease. Elevated haptoglobin (Hp) and serum amyloid A (SAA) have been observed to correlate with increased leukocyte and inflammatory markers, indicating that Hp and SAA may act as mediators of the host inflammatory response. Further, there are ironic roles of APPs, such as the role of fibrinogen in tissue repair and infection control, which can also result in pathological changes, including fibrin deposition and chronic inflammation. Cranioventral consolidation and necrotic lesions in infected cattle are further supported by histopathological observation in affected tissue, explaining the role of APPs and the relation between tissue damage and disease progression (Xu et al., 2022).

APPs as Diagnostic and Prognostic Tools

APPs are important diagnostic and prognostic tools in veterinary and human medicine, reporting an inflammatory response of the body. Sensitive APPs (C-reactive protein (CRP) and serum amyloid A) are excellent markers of inflammation, outperforming the conventional white blood cell count as a marker of it (Güleç et al., 2022). There is a particular utility of APPs for differentiating conditions such as feline infectious peritonitis (FIP) from other conditions in veterinary practice with overlapping symptoms. Measurement of these proteins in a number of biological fluids improves diagnostic capabilities in many disease states (Rossi, 2023). Table 2 presents the comparison of different testing methods for diagnostic. They also serve as a prognostic indicator of disease progression or therapeutic response, and profiling such apps proved useful in monitoring chronic diseases (Stanke et al., 2023). Even specific APPs, namely lactoferrin, have also been shown to be implicated in neurodegenerative diseases, such as Alzheimer's, and used to predict disease severity and progression (Tsatsanis et al., 2021). Nevertheless, the diagnosis of APPs requires careful interpretation of the non-specific nature of APPs with clinical examination, and additional research may contribute to augmenting diagnostic precision with more specific markers (Stanke et al., 2023).

Table 2: Comparison of Diagnostic Methods for *M. bovis*

Method	Advantages	Limitations	Field Applicability	References
APP Testing	Rapid, less invasive	Non-specificity	High (portable kits available)	(Cannon, 2024)
PCR	High specificity	Expensive, needs equipment	Limited	(Deborah M Cooke et al., 2024)
Culture	Accurate, gold standard	Time-consuming	Low	(Ferrari et al., 2024)

Serum concentrations of both haptoglobin (Hp) and serum amyloid A (SAA) are upregulated in calves infected with *Mycoplasma Bovis* when compared with a healthy control group, which suggests that they are part of the APPs (Sameed Saher et al., 2024). Measuring APP levels is diagnostic; especially in subclinical cases, and has improved livestock management consequently. Plasmatic APP is also upregulated in infected calves, but, alongside it, oxidative stress markers are also enhanced, pointing to a complex relationship between inflammation and oxidative perturbation (Saco & Bassols, 2023). However, the general nature of APPs for diseases implies that a more extensive diagnostic evaluation of clinical assessment and additional laboratory tests must be employed.

Diagnostic Applications of APPs for *M. bovis* Infections

Current Diagnostic Methods

The identification of *M. bovis* infections has in the past been achieved using the culture method, PCR, and serological tests. Culture is the most accurate test to prove *M. bovis* infection, but it is also the slowest because the bacterium is slow-growing, taking several weeks for results. Whereas, PCR is specific and fast, belonging to the limitation by sampling and the existence of inhibitors in the multisampling biological phenotypes (Song et al., 2024). Several biochemical tests, including enzyme-linked immunosorbent assays (ELISAs), can measure antibodies against an infectious agent; however, the tests can be insensitive in animals with subtle clinical signs of infection (Gomes et al., 2024). In addition, serological tests are frequently unable to differentiate between ongoing or recurrent disease and old exposure, which is an issue in monitoring and containing the spread of diseases. Concisely, these existing diagnostic tools have their shortcomings for speed and sensitivity, practicality especially in field conditions or in subclinical infection.

Limitations of Existing Diagnostic Tools

Challenges associated with a diagnosis of APPs during *Mycoplasma Bovis* infections in small ruminants using other diagnostic tools have various aspects. Even though the proteins of APPs such as, serum amyloid A and haptoglobin can offer much information about an animal's health condition, these are not specific enough in order to identify certain infections, including *Mycoplasma Bovis*. These and other difficulties relate to the lack of differentiation between the many forms of neuropathy and the ways in which the diseases manifest themselves. APPs are upregulated in various pathogens infections so they cannot be used to single out *Mycoplasma Bovis*. The convergence of the responses of APPs to varying pathogens complicates the analysis of the results (Reczyńska et al., 2018). The current diagnostic tools that are widely used include the serological assays and PCR and these cannot be easily used in field settings due to the equipment required. The high cost of applying refined procedures such as mass spectrometry constrains the applicability of the techniques. It also has implications for late seroconversion in APPs whereby the infections by *Mycoplasma Bovis* will not be identified early (Okella et al., 2023). Despite its high effectiveness, PCR suffers from high cost and its need for highly specialized equipment, making it unsuitable for resource-challenged settings. Serological tests are, however, known to mimic reactions from other pathogens, and this gives false-positive results (Fasogbon et al., 2024). However, none of the above methods identifies subclinical or chronic infections, which are essential for monitoring the health status of stocks in a herd and controlling disease spread. These restrictions imply the necessity of developing other types of diagnostics that are rapid, cheap, and that allow the identification of infections at any stage of the disease.

Advantages of APP-Based Diagnostics

APPs, including Hp and SAA, are considered to have essential advantages for the diagnosis of *Mycoplasma Bovis* infection in small ruminants. It's fast rise upon infection allows one to identify both early and non-specific, subclinical, or asymptomatic process, which are still undetectable within the scope of standard diagnostic approaches (Iliev & Georgieva, 2019). The early detection is followed by necessary measures that help to minimize the possible economic damages from *M. bovis* infections which have severe potentially negative consequences for the productivity of livestock (Sameed Saher et al., 2024). Furthermore, the upgrading of APPs is useful for chronic infection due to the fact that those elevations persist in the course of inflammation and infection. They can also help in differentiating between bacterial and viral infections, a choice of appropriate treatment, and control the effectiveness of management interventions (Iliev & Georgieva, 2019). Hence, routine use of APPs is feasible but suboptimal because they give valuable information on the animals' systemic inflammatory state while being non-specific in their functionality due to which they should be used in combination with other diagnostic modalities. This complementary approach improves the assessment of *M. bovis* detection as well as its reliability.

Field Applications of APPs Testing

Due to the fact that APP testing is conducted in the field, it proves to be useful for veterinarians as well as livestock managers. For example, point-of-care testing for Hp and SAA is simple, rapid and can be done on farms without necessitating the transfer of animals. These tests are particularly useful for culling, sample testing on presumably infected animals, and investigating controls in a herd over a period of time. Besides using for individual animal diagnostics, APP testing may serve herd health management by giving information on the general status of inflammation of the lactating cow herd for that particular season. With this information, successful management practices can be directed like, vaccinations, suitable antimicrobial treatments, and biosecurity methods, to prevent diseases. The fact that APP's testing tools are portable and intuitive is also advantageous when it comes to usability in the field to overcome the practical difficulties of a conventional testing approach (Paksoy et al., 2024).

Integrating APPs into Multiplex Diagnostic Platforms

The incorporation of APPs into multiplex diagnostics systems is an important advancement in the diagnostics of infectious and inflammatory diseases. Commercial platforms with multiple biomarker potentiality, for instance, the Bio-Plex Pro system, measures several biomarkers, including C-reactive protein (CRP), Hp and SAA (Tor et al., 2024). This helps to improve diagnostic performance because it furnishes all the requisite data concerning the inflammation state of individuals within a single test. The combination of magnetic bead technology and the skilled application of washing systems also enhances the throughput and reduces variability, which finally enhances the reproducibility of these platforms especially for high-throughput testing. In veterinary diagnosis, multiplex assays for APPs have been useful in the diagnosis of infections in stock and small ruminants, including *Mycobacterium bovis* (Molina-Moya et al., 2023). APP level tracking makes it possible to note changes and make interventions in order to prevent disease outbreaks within a specific herd. By assessing multiple APPs at the same time, these platforms provide clinicians with comprehensive evaluations of inflammatory processes, facilitate the distinction of diseases, and provide suggestions for the right treatments.

Challenges and Future Perspectives

Since APPs pose a tremendous diagnostic prospect in determining *Mycoplasma Bovis* infection, there are a number of drawbacks that restrict their usage regularly in the absence of other markers. This fact indicates that the fact that APP can increase in response to various inflammatory processes, including stress, concomitant infections, and non-bacterial diseases, does not allow specifying the cause of elevated levels of these markers only by *M. bovis*. In addition, the differences in APP levels because of variations in the animal physiology and concurrent diseases make the diagnosis difficult, which highlights the importance of using APP testing in combination with other diagnostic techniques to improve the sensitivity of the test. There is an added concern about how APP is measured and how their scores are interpreted, because there is no universal standard for doing these assessments. Due to the lack of standard thresholds that qualify APP as raised in SRs, their diagnostic usefulness and general applicability are rather limited. Greater specificity of reference values according to species and production type, as well as routine monitoring of the interlaboratory and interfiled assay variability, would be crucial for increasing confidence in APP-based diagnostics. In spite of these challenges, APPs present a great potential for the promotion of disease management. Apart from diagnostic purpose, APP levels are potential biomarkers for the evaluation of therapeutic approaches, disease severity, and vaccines. Their use can help to yield insight as to the resolution of infections or immune activation and as a result, help in the assessment of therapeutic interventional and vaccine efficacy.

Future Directions

Further studies should be devoted to the identification of a 'new' realistic APP with high sensitivity and specificity for the detection of *M. bovis* in small ruminants. Therefore, the identification of other APPs or other biomarker profiles could improve diagnostic accuracy and may give more detail regarding disease pathophysiology. Another promising direction of the invention is the creation of new real-time field-applicable APP tests that can provide rapid diagnostics in the conditions of the limited availability of resources. Last but not least, the incorporation of APP testing into 'precision veterinary medicine' paradigm could potentially transform livestock production through precision livestock management.

Conclusion

The management of *Mycoplasma bovis* infections in small ruminants depends heavily on APPs for early disease detection while also monitoring disease progression as well as assessing treatment responses. Systemic inflammation shows high sensitivity through Hp and SAA markers, which helps diagnose active and subtle infections during resource-constrained conditions when standard diagnostic methods prove difficult. The flexibility and speed of APP test analysis for detecting diseases allow substantial widespread disease control through simple portable testing methods. Non-specific binding of APP to proteins represents their main drawback since this biomarker can increase due to diverse factors including stress and other infection types. The specificity of APP testing improves when researchers combine it with additional diagnostic methods. The successful implementation of APP testing requires improvements in reference range establishment along with standardized protocol development and consistent assay performance standards. Biomedical applications combining APP testing with multiplex diagnostics and precision animal healthcare show great potential to transform *M. bovis* disease management techniques. Upcoming medical developments incorporating field-based rapid APP test technology with higher-specific biomarkers will boost assessment precision. APPs demonstrate the potential to enhance vaccine performance and treatment surveillance which leads to reduced economic impact and improved animal well-being and increased production value.

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