Academic Journal of Animal Diseases 11(3): 53-58, 2022
ISSN 2079-200X

DOI: 10.5829/idosi.ajad.2022.53.58

A Review on Diagnostic Methods of Contagious Caprine Pleuropneumonia (CCPP)

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Abstract: Contagious caprine pleuropneumonia (CCPP) is a serious respiratory disease of domestic goats and some wild ruminants caused by *M. capricolum* subsp. *Capripneumoniae* (Mccp). A diagnosis of CCPP is imperative for timely detection and devising interventions that prevent disease spread and loss to farmers. Diagnosis of CCPP involves clinical signs, gross morphological lesions on postmortem, histopathology, culture and isolation, hematological, biochemical, serological and molecular diagnostic tests. Culture and isolation confirm the causative agent disease *Mycoplasma capricolum* subsp. *capripneumoniae*. Serological tests are available that are used in the field for the confirmatory diagnosis of CCPP. The latex agglutination test which detects serum antibodies in CCPP-infected goats is more sensitive and can be performed in field conditions using whole blood or undiluted serum with a prompt result. An indirect enzyme-linked immunosorbent assay (ELISA) has been developed to screen goat serum at a single dilution of antibody to *Mccp*. The specificity and suitability of ELISA for large scale testing make it an appropriate tool for epidemiological investigation of CCPP. Direct and indirect fluorescent antibody tests are the simple, reliable and rapid serological methods applied to clinical samples for the identification of most *Mycoplasma*s. Whole genome sequencing has become the gold standard for high resolution typing method that replace all phenotypic or genotypic methods.

Key words: Contagious Caprine Pleuropneumonia · Culture · Diagnosis · Enzyme Linked Immunosorbent Assay · Polymerase Chain Reaction

INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) is a serious respiratory disease of domestic goats and some wild ruminants caused by M. capricolum subsp. Capripneumoniae (Mccp). Contagious caprine pleuropneumonia (CCPP) is a devastating disease of goats first described in 1873 [1]. After a century, the causal agent was isolated in 1976 in Kenya from the lungs of goats [2]. It is a listed disease of the World Organization for Animal Health (OIE) that causes a huge economic loss for goat producers [3]. Mycpolasma capricolum subsp. capripneumoniae (Mccp) is the causative agent of CCPP. Previously, this agent was known as Mycoplasma sp. type F38 [4], a disease of domestic goats [5] and affects some wild ruminants [6] is a highly contagious and serious respiratory disease.

It is characterized by coughing with nasal discharge, severe respiratory distress, difficulty walking, high morbidity (80-100%) and the disease spreads inevitably to the whole flock. In the absence of treatment, mortality is also very high and may reach 60-80% [7]. The lesions at necropsy are mainly fibrinous pleuropneumonia with unilateral hepatization and accumulation of straw-colored pleural fluid [8].

CCPP is a major threat to goat farming in parts of Africa and Asia [9] and over 330 million poor people keep the livestock [10]. Still the exact distribution of CCPP is not known and represents a significant threat to many disease-free countries [3]. There have been very few declarations of CCPP outbreaks to the OIE in the previous years due to lack of awareness of this disease and possible confusion with other diseases, such as peste des petits ruminants (PPR) and Pasteurellosis [11]. Mccp has been isolated in 20 countries and clinically described in nearly 40 countries globally [12].

To diagnose CCPP a number of serological tests are available including CFT, LAT, GIT and cELISA. PCR method has radically improved the detection and identification of Mccp which do not grow easily in-vitro [1]. Therefore, the objectives of this paper are to review diagnostic methods that are used for screening, isolation, monitoring or epidemiological surveillance and confirmatory for Contagious caprine pleuropneumonia.

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Tentative Diagnosis: A tentative diagnosis of CCPP can be given based on clinical signs but confirmation of the disease is difficult for reasons that, from clinical point of view, CCPP cannot be differentiated from a number of diseases presenting similar respiratory signs in small ruminants, such as Peste des petits ruminants and pasteurellosis, thus laboratory confirmation is required for differential diagnosis with other diseases [13]. However, being fastidious organisms, it is very difficult to isolate Mycoplasmas on ordinary media in in vitro culture. In fact, it has been observed that a negative bacteriological result does not indicate the absence of infection [14].

An accurate and reliable diagnostic technique is essentially required for rapid detection and confirmation of infected animals [15]. Prompt diagnosis is crucial for effective disease control and monitoring. However, the spread and burden of CCPP remain largely unknown, mainly due to inadequately funded veterinary services, an absence of infrastructure enabling swift sample transport and the lack of rapid, inexpensive, sensitive and specific diagnostic tests applicable for field use [16].

Confirmatory diagnosis is based on the isolation of Mccp from clinical samples of lungs or fluids [12]. Whole genome sequencing has become the gold standard for high resolution typing methods, which supersedes all previous phenotypic or genotypic methods, which could be applied for major public health pathogens [17].

Culture and Isolation from Appropriate Sample: The sample of choice from affected animals is the pleural fluid, which contains high numbers of mycoplasmas and sections of hepatized lung preferably at the interface between normal and diseased tissue. Samples must be sent quickly in a cool condition but will become of little value if journey time is longer than 2 days. Sending frozen samples is recommended but not always practical [12]. During the investigation of CCPP in Eritrea, excellent isolation rates of Mccp were achieved from lyophilized lung samples even though isolation was carried out only several weeks after arrival [18].

The isolation of Mccp is difficult because of its highly fastidious nature [12]. A number of media have been used for the general growth and isolation of Mycoplasma. Mycoplasma agar and broth media (Oxoid; Sigma), are used for the selective isolation of Mycoplasma spp. An agar non-selective media under the product code name CC1A (Mycoplasma Experience Ltd. Product), is available that allows the development of Mccp as red colonies over seven days of incubation. Mccp has been successfully grown and isolated from infected lungs through culturing on Hayflick medium broth H25P [19].

Similarly modified Hayflicks media have been used for the growth and isolation of Mccp organisms [3]. Other than Mccp (five to seven days in vitro growth), all Mycoplasma mycoides cluster members grow within 24-48 h in vitro, producing colonies 1-3 mm in diameter [20]. On agar media, under inverted microscope Mccp colonies have the typical fried egg appearance observed in other mycoplasmas [19].

For preliminary screening, a limited number of biochemical tests are available based on nutritional capabilities of Mccp or specific enzyme activities [9]. Digitonin sensitivity distinguishes Mycoplasmas from acholeplasmas and serum digestion distinguishes members of the Mycoplasma mycoides cluster from all other small ruminant Mycoplasmas [21]. Phosphatase production separates Mcc from other members of the Mycoides cluster, while metabolic differences (such as maltose positive reaction for Mccp) allow differentiation between Mcc and Mccp[22]. The interspecies variation in some biochemical reactions is often remarkable, rendering their application valueless. The lack of arginine catabolism in Mccp may help to differentiate it from Mcc[19], but in some strains of Mcc arginine catabolism is reported to be lacking or very difficult to detect [15].

Immunological Test

Complement Fixation Test (CFT): Complement fixation test (CFT) the prescribed test for international trade, demonstrating the potential limitations of antibody detection as a sole diagnostic technique. CFT expected to induce cross-reaction to the mycoides cluster due to use of a crude antigen. Seroconversion of Mccp was observed by CFT to start 7–9 days after the appearance of clinical signs, peak between days 22 and 30 and decline rapidly thereafter [23].

Latex Agglutination Test (LAT): LAT for antibodies detection uses capsular polysaccharide specific antigen (CPS) coated beads extracted from Mccp, which agglutinate in the 20 presences of specific antibodies in the blood of affected goats [24]. It can be run in two minutes on samples of whole blood or serum, without the need for any specialist training or sophisticated equipment and is adaptable to any laboratory or field conditions. It's specificity is not well characterized. Cross-reactions may occur as Mccp polysaccharides are similar to those produced by M. leachii and may be found in other bacteria. It is a very useful test in an outbreak because it can be performed at the pen side using a drop of whole blood. This test is sensitive at an early stage of the disease as long as IgM persists in the serum.

The test is carried out by mixing a drop of the sensitized beads with a drop of blood or serum from the suspected animal on a glass slide for one minute and the results read visually and recorded as positive or negative [25].

Growth Inhibition Test (GIT): GIT is the least sensitive and simplest of the tests available for CCPP diagnosis (OIE, 2008). It depends on the direct inhibition of growth of Mycoplasma on solid media by specific hyperimmune serum and detects primary surface antigens. The GIT is particularly useful in identifying Mccp because they appear to be serologically homogeneous and antiserum to the type strain produces wide inhibition zones [25]. GIT is the least sensitive and simplest of the tests available for CCPP diagnosis [25]. It depends on the direct inhibition of Mycoplasma growth on solid media by specific hyperimmune serum and detects primary surface antigens. The GIT is particularly useful in identifying Mccp because they appear to be serologically homogeneous and anti-serum to the type strain produces wide inhibition zones [25].

Enzyme-Linked Immunosorbent Assay (ELISA): It is a competitive enzyme-linked immunosorbent assay based on the monoclonal antibody Mab 4.52. Mabs were produced by immunizing mice with adjuvant mycoplasma antigen and standard fusion cloning procedures [26]. cELISA could be used as a surveillance tool in CCPP free regions at risk of disease introduction, including all the regions bordering infected zones. ELISA is likely to be more suitable for epidemiological surveillance and seroprevalence studies. ELISA was modified to produce a heat-stable laboratory diagnostic kit suitable for prevalence and vaccine efficacy screening [11] and it is a newly developed test, which permits the specific detection of antibodies in animals, which have been affected by CCPP. This test is based on the use of a monoclonal antibody (MAb), which is competing with goat antibodies to bind to the antigen that is coated on the plates. The specificity of the test depends on the epitope that is recognized by the MAb. The introduction of the cELISA for CCPP will permit the implementation of serological studies on a large scale [27]. However, it is not suitable for detecting acute disease in the field; because sero-conversion appears 2-3 weeks after infection and acute cases lead to death before insurgence of antibody response. The high specificity of cELISA close to 100% was similar to the blocking ELISA. The high cutoff point, maximize the diagnostic specificity but it is expected to decrease diagnostic sensitivity of the test [11].

Competitive Enzyme Linked Immunosorbent Assay: A C-ELISA was developed [28] and proved both specific and sensitive. This test has recently been reformatted as a kit containing pre-coated plates and ready-made reagents, including MAb 4/52. It is now a strict competition assay instead of a semi-blocking test as in the original publication. The new kit has been re-validated to establish its cut-off value, 55% inhibition (PI), to obtain a strict specificity of 99.9%. It allows the detection of positive sera in CCPP-infected herds, but its true sensitivity at the individual level has not yet been fully evaluated. As it is highly specific, it can be used to evaluate herd status using targeted sampling of recovered animals in the tested herds should greatly enhance the sensitivity without any specificity problem. In the OIE Reference Laboratory, the uncertainty of measurement for this C-ELISA has been evaluated at ±8 PI. This test can be used to evaluate the CCPP vaccine quality as the seroconversion measured 1- and 2-months post-vaccination is proportional to the Mccp antigen or saponin content. However, the correlation between C-ELISA titre and protection has not yet been established [11].

Direct and Indirect Fluorescent Antibody Tests: These are the simple, reliable and rapid serological methods applied to clinical samples for the identification of most Mycoplasma species [26]. Among many, the indirect fluorescent antibody (IFA) test is the most commonly used and is applied to unfixed Mycoplasma colonies on agar [25].

Dot Blot Immunoassay: A 5-µl aliquot of mycoplasma culture was dropped on pieces of hybridization transfer membrane, left to dry at 37°C for 10 min, washed three times with tris buffer solution (TBS), incubated with blocking buffer (TBS + 10% horse serum) for 30 min and later with Mycoplasma capricolum subsp. Capri-pneumoniae specific monoclonal antibody. After three washings, immuno-enzymatic antigen antibody reaction was determined using anti-mice immunoglobulin G conjugate with horseradish peroxidase (HRP, DAKO), which gave a brown staining following reaction with orthophenylene diamine (OPD) chromogen and hydrogen peroxide substrate [26].

Molecular Diagnostic Tests: Until recently, isolation was the only way to confirm the presence of CCPP. A DNA probe that differentiates Mccp from other members of the Mycoplasma mycoides cluster was developed. PCR-based

diagnostic systems are used for the rapid detection, identification and differentiation of the Mycoplasma mycoides cluster members to the serovar and strain levels [16]. Species identification based on PCR of the 16S rRNA genes and restriction at positions where unique differences occur between the two operons has been demonstrated previously for Mccp[26].

Because of antigenic and genetic similarities among the member of the mycoides cluster, the best and most accurate diagnostic method for the identification of Mccp is molecular typing [29]. Recently a field-applicable recombinase polymerase amplification assay for rapid detection of Mccp has been developed [16] All members of the M. mycoides cluster have two rRNA operons and there are differences in the sequence of 16S rRNA genes of the two operons. Many of the members of Mycoplasma share genomic and antigenic structures that often cause immunological cross-reactions.

However, different species can be distinguished from each other by using different molecular techniques [14, 27]. PCR method has radically improved the detection and identification of microorganisms that do not grow easily in-vitro. The tests have been described and shown to be specific, sensitive and can be applied directly to clinical material, such as lung and pleural fluid, dried samples on filter paper and culture material [17]. Due to the difficulty of isolating Mccp, PCR is the technique of choice for the diagnosis of CCPP.

Sequencing: Sequencing of the gene for 16S ribosomal RNA has also been used to develop a PCR-based test where the final identification of Mccp is made depending on the pattern of the products after digestion of the PCR product with the restriction enzyme Pst1 [30]. An improved resolution method, MLSA (multi-locus sequence analysis) based on the analysis of several genetic markers has also been used for the identification of Mccp[3]. Sequence-based genotyping methods for bacterial typing are technically simple, objective oriented and portable. Moreover, they allow direct amplification and sequencing of the organism from clinical material [3].

CONCLUSION

Contagious caprine pleuropneumonia (CCPP) is caused by *Mycoplasma capricolum*subsp. *capripneumoniae*(*Mccp*) which belongs to the *Mycoplasma mycoides* cluster, a group of five closely related *Mycoplasma*s, pathogenic to ruminants. It has been noted that every year outbreaks of respiratory diseases occur in the goat population with an alarming rate of mortality but the lack of advanced techniques is a big hindrance in the proper diagnosis of CCPP. Diagnosis of the disease is one of the challenging aspects as it influences prophylactic and therapeutic regimens and the control strategies for the prevention of global spread. Culture of Mccp, though a gold standard, is very difficult, lengthy and costly process and is not a very realistic approach that can have widespread use. It is also difficult to rely on serology for the member of the Mycoplasma mycoides cluster in goats due to the frequent occurrence of cross-reacting of antibodies. Such tests are best used on a herd basis rather than for diagnosis in individual animals. The complement fixation test (CFT) remains the most widely used serological test for CCPP. In CCPP, the complement fixation test is the recommended test for detection of Mycoplasma capricolum sub species capripneumoniae infection. PCR method has radically improved the detection and identification of microorganisms which do not grow easily in-vitro. The tests have been described and shown to be specific, sensitive and can be applied directly to clinical material, such as lung and pleural fluid, dried sample on filter paper and culture material. Therefore, based on the above conclusive remarks the following recommendations are forwarded: The development of simples, accurate and cheaper diagnostic tools will be helpful for efficient surveillance, detection and effective control of CCPP. It is the responsibility of the livestock industry to implement advanced technologies for the molecular typing of the causative agent of CCPP or CCPP-like diseases in goats in Ethiopia.

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