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Diagnostic Techniques of Contagious Caprine Pleuropneumonia: A Review

Abdela Bulbula Ogeto*

Department of Bacterial Serology, Animal Health Institute, Sebeta, Oromia, Ethiopia

SUMMARY

Contagious caprine pleuropneumonia is an important disease of caprine in different parts of the world and can cause huge mortality in immunologically naive herds. Currently available methods for the diagnosis of contagious caprine pleuropneumonia include; clinical signs, postmortem examination, cultivation, serological assays, and molecular assays. Post-mortem examination reveals fibrinous pleuropneumonia with massive lung hepatization and pleurisy, accompanied by an increase of straw-colored pleural fluid. Confirmatory diagnosis is based on the isolation of *Mycoplasma capricolum* subspecies *capripneumoniae*. Serological tests to detect antibodies to this organism include complement fixation, latex agglutination, and competitive enzyme-linked immunosorbent assays. The complement fixation test remains the most widely used serological test for Contagious caprine pleuropneumonia, although the latex agglutination test is being progressively more used in diagnostic laboratories as well as a pen-side test. A specific competitive enzyme-linked immunosorbent assay has been developed but is not widely available. Thus, for diagnosing Contagious caprine pleuropneumonia, the gold standard test is the direct isolation and cultivation of *M. capricolum* subsp. *capripneumoniae*. Polymerase chain reaction-based tests have been described to be specific, sensitive and can apply to clinical material. In general, the present polymerase chain reaction and sequencing have been used to set up the molecular epidemiology of contagious caprine pleuropneumonia. Hence, the objective of this topic is to assess diagnostic techniques for contagious caprine pleuropneumonia. In conclusion; postmortem examination, latex agglutination, and polymerase chain reaction techniques are used, especially in specificity to confirm Diseases in outbreak and to help control rapidly. Despite difficulties in diagnosis focus on devising good and skilled technical expertise, and funding for proper diagnostic facilities in developing countries, including Ethiopia with a particular focus on both conventional diagnostics and advanced serological tests.

Keywords: contagious caprine pleuropneumonia, diagnostic tests, small ruminants, isolation, diagnosis

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***Corresponding Author**

Abdela Bulbula Ogeto

Department of Bacterial Serology, Animal Health Institute, Sebeta, Oromia, Ethiopia.

Tel: +251910398915

E-mail: abdallahbulbula@gmail.com

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INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) is an important disease of caprine in Africa, the Middle East and parts of Asia, and can cause mortality of up to 80% in immunologically naive herds (Soayfane et al., 2018) [1]. In Ethiopia, the presence of CCPP was confirmed in 1990 following the isolation of *M. capricolum* subsp. *capripneumoniae* (Mccp) from outbreaks (Thiaucourt et al., 1996) [2]. In addition, the disease was registered as a member list of notifiable diseases of the world organization for animal health (OIE, 2008) [3], due to its significant role in causing large animal population deaths, especially goats where it is endemic.

The etiology of CCPP is by so-called Mccp, which was classified under *Mycoplasma mycoides* clusters (Liljander et al., 2015) [4]. The disease is mainly confined to the thoracic cavity and transferred from one animal to another through contact by the in-breathing of droplets from infected animals (Nicholas et al., 2018) [5].

The diagnosis of contagious caprine pleuropneumonia is highly complicated with another respiratory system diseases such as; contagious agalactia syndrome, also known as mastitis, arthritis, keratitis, pneumonia, and septicaemia syndrome (MAKEPS) and pasteurellosis. On-time diagnosis is essential for effective disease control and monitoring. Thus it needs appropriate diagnostic techniques to facilitate the success of control and prevention of the disease (Nicholas et al., 2019) [6].

Diagnostic methods of (CCPP) mainly depend on clinical signs and post-mortem findings. But, the likeness of clinical signs by different species made the major challenge of diagnosis techniques (Kalshingi et al. 2015) [7].

Pleural fluid was described as the sample of choice. Although, recent reports from an experimental infection indicate detection as low as to 25% in it and high in the lung tissue (Schnee et al., 2011) [8].

Laboratory diagnosis includes sampling protocol, expensive but highly specific media and culturing techniques, safe laboratory, identification and characterization by specific and sensitive methods involving colony characteristics, staining, morphology, biochemical testing, agglutination, complement fixation, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and genome sequencing (Yatoo et al., 2018) [9]. Although, Laboratory diagnosis of the disease does not amount to its importance in some countries and this is mainly due to the insufficiency of laboratory facilities, experience and difficulty to isolate the bacteria (Saeed & Osman, 2018) [10].

Isolation of Mccp from the lung tissue and pleural discharge of infected shoats is used as definitive diagnosis of CCPP. The diagnosis techniques such as; biochemical, immunological and molecular have been more important role in the identification of CCPP isolates of infected animals. As recommended by Office International des Epizooties (OIE) for international trade, complement fixation test (CFT) and the competition ELISA are preferred serological methods for detecting specific antibodies of contagious caprine pleuropneumonia species (Francis et al. 2015) [11].

The Biochemical assays of the local isolates are carried out for identification of the species of the mycoplasma cluster as per standard protocol. The biochemically identified samples of the species of mycoplasma Mccp are subjected to deoxyribonucleic acid (DNA) extraction for confirmation through PCR (Adehan et al., 2006) [12].

In general, isolation of the organism from clinical samples of lung and pleural fluid of infected goats/sheep is more appropriate diagnostic method used for confirmation.

In addition, serological tests like indirect hemagglutination, CFT, and latex agglutination (LAT) are commonly used to detect the antibody response of goats to Mccp (Samiullah, 2013) [13]. On the other hand, the competitive enzyme-linked immunosorbent assay (cELISA) for CCPP has been developed and found highly specific (Teshome et al., 2019) [14]. Recently, some PCR and real-time PCR methods have been developed and found specific and efficient in the detection of Mccp and recommended for confirmation of clinical signs (OIE, 2014) [15]. Therefore, the general objective of this topic is: to assess the diagnostic techniques for contagious caprine pleuropneumonia and specifically, for understanding the better diagnostic techniques, in order to identify the organism from other species of Mycoplasma to control and vaccination.

LITERATURE REVIEW

Diagnostic techniques of contagious caprine pleuropneumonia

Diagnosis of CCPP has often been considered difficult due to the existence of complications with other diseases which can cause confusion as symptoms and lesions are often

overlapping. Isolation of Mycoplasma is unwieldy and too slow to be of any practical use in the containment of the outbreaks. The diagnostics techniques (tests) comprise; clinical signs, postmortem, bacteriological (isolation), and molecular (PCR) as listed generally by World Organization for animal health (OIE, 2015) [16].

Confirmatory diagnosis is described as the isolation of Mccp from clinical samples of diseased lung and pleural discharges. The ideal sample for Mccp isolation is pleural fluid obtained from a recently slaughtered or live-infected goat. Unlike truth CCPP caused by Mccp, other Mycoplasma infections can spread beyond the chest cavity. To overcome these constraints among different species, DNA amplification techniques provide accurate identification of *M. mycoides* cluster members (Nicholas et al., 2019) [6].

Clinical diagnosis and postmortem examination

Clinical signs and post-mortem lesions are the main methods routinely used for diagnosis of CCPP in the area of study, which are not sufficient to establish the diagnosis of CCPP (Saeed & Osman, 2018) [10]. Pleurisy is escorted by increase in number of straw colored pleural fluids; a lung covered with thick yellow coat of fibrin and showing pleural adhesion, a lung showing congestion and red hepatization and larger in size compared to the non-infected one and straw colored Pleural fluids as indicated in (Figure 1).

The clinical signs of the disease occur in three different phases. The first phase is known as peracute and characterized by the diseased animals in one to three days after infection. The second phase is acute. In this, increment of body temperature (up to 43°C), anorexia, lethargy, and frequent coughing. Also, inability to walk, standing by front legs, frothy nasal fluids, abortion, stiffness of necks, and death in seven to 10 days can be observed at this acute phase. Chronic: there is chronic cough, nasal discharge, and debilitation (Nicholas et al., 2019) [6].

Both the diseased and control goats should euthanized by throat cutting without breaking the neck and thoroughly examined post-mortem. If present, lung consolidation, pleural fluid accumulation, fibrinous pleurisy, pericardial fluid accumulation were recorded and described (Aklilu et al., 2015) [17].

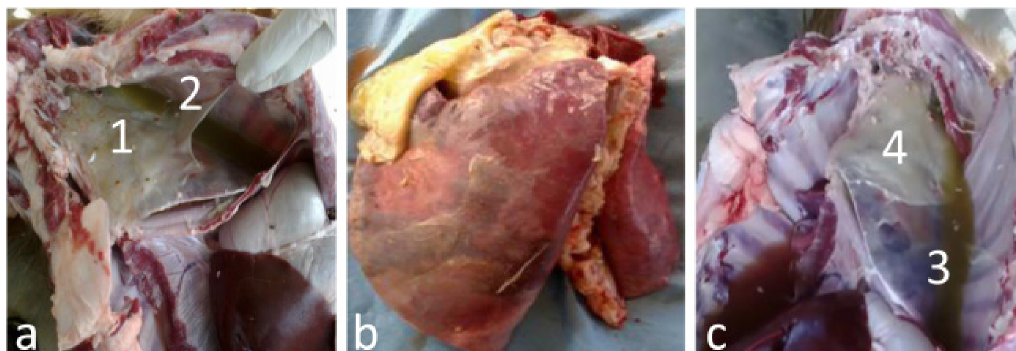

Figure 1: Postmortem findings of goats infected with contagious caprine pleuropneumonia (a) a lung covered with thick yellow coat of fibrin (1) and showing pleural adhesion (2); b) a lung showing congestion and red hepatization and larger in size compared to the non-infected one; c) straw-colored Pleural fluids (3). Note lung fibrin coat (4).

Culturing

Different types of growth medias (including agar or broth) are important for isolation of Mccp. Although, pleuropneumonia like organism (PPLO) agar (broth) is mostly used and selected for purpose of isolating the organism in laboratory under sterile condition from the culture of nasal discharges and pleural fluids (El-Deeb et al., 2017) [18].

In addition, the culturing or isolation of Mccp needs specialized medium for development, advanced laboratory capacity and long first incubation period up to the minimum of five to seven days with average of four days at 37°C, having 5% carbon dioxide in the presence sterile laboratory state. During isolation; pleural fluid, lung tissue or pleural tissue, and nasal discharges are important samples and also, Giemsa

or Diene's stain supports the identification. Moreover, the agent identification is processed by Diagnostic methods such as biochemical tests, immunological (Serological), and molecular tests (Gene-based) ((Francis et al., 2015) [11].

Biochemical Tests: Based on its specificity, reliability and having good quality, molecular diagnostic tests are preferred for the diagnosis of CCPP. Thus, these modern molecular methods represented the biochemical tests in present time (OIE, 2014) [15].

Serological tests

Serological tests to detect antibodies to Mccp include CFT, LAT, which can identify early immune-globulin M (IgM) antibodies, and cELISA. Animals with acute CCPP rarely develop measurable titers before death; antibodies usually become detectable 7-9 days after the first clinical signs. Whenever possible, paired serum samples should be collected 3-8 weeks apart. Serological tests are generally used on a herd basis and not for individual diagnosis. These tests do not identify all reactors, and cross-reactivity is an issue. The newly modified a cELISA for CCPP is to generate a heat stable laboratory diagnostic kit appropriate for prevalence and vaccine efficacy screening and not to cross-react with other Mycoplasma found in goats; though, it is not suitable for detecting acute disease in the field (Peyraud et al., 2014) [19].

Thus, for diagnosing CCPP, the gold standard test is the direct isolation and cultivation of MCCP from infected lung tissues or pleural fluid collected postmortem. The above different explanations, shows that serology should be applied on a herd, not an individual basis, and that whenever possible, paired serum samples collected 3-8 weeks apart, should be examined (Liljander et al., 2015) [20].

Complement fixation test (CFT): This test was applied as better diagnosis technique for identification of CCPP in 1983 by Muthomi and Rurangirwa [21]. Now a day, the method is widely serving in areas of international trade as illustrated by Nicholas et al. [1].

In number of studies reviewed to estimate prevalence of the CPPP, the CFT is the predominant diagnostic techniques used followed by ELISA. CFT findings emphasize the difficulties inherent in the serological diagnosis of CCPP when using whole cell or membrane preparations as antigen. The use of the more defined antigen, the polysaccharide elaborated by Mccp, provides greater specificity, as there is no cross-reactivity with sera against the other three principal *caprine mycoplasmas* (Asmare et al., 2016) [22].

Latex agglutination test (LAT): This test differs from other serological diagnostic techniques by being easy, fast and can be done outside laboratory during outbreak case. By using the drop of serum or whole blood of infected sheep and goats, Mccp antibody can be simply identified on LAT slide (Nicholas et al. 2018) [1].

Competitive enzyme-linked immunosorbent assay (cELISA): This technique was recommended and applied by Thiaucourt et al. [23] in 1994 for diagnosis of CCPP to enhance immunological research in huge number of population. Thus, the application of this assay for diagnosis CCPP is better choice by using random/clinical history based sampling methods to develop the chance sensitivity without losing specificity (More et al. 2017) [24]. But, it has absence of capacity in detecting antibody of the pathogen at the beginning stage (Jean de Dieu et al., 2019) [25].

Polymerase chain reaction (PCR) test

Is specific and sensitive test that was selected as the better diagnosis method for detection of CCPP from pleural fluid and tissue samples of infected animals (OIE, 2017) [26]. In 1994, Bascunana C, et al. [27] discovered the PCR assay which amplifies the 16S rRNA gene of the *mycoides* cluster and on the other hand, PCR method which was specific in amplification of Mccp supported with primers sequences specific for this strains was developed by Woubit S, et al. [28] in 2004 (Teshome & Sori, 2021) [14].

For instance, the so called Multi Locus Sequence Analysis (MLSA) which depends on and has ability to analyses huge number of genetic markers has been shared great roles in identification of Mccp. Also, another genotyping technique which is depend on sequencing is advanced diagnosis methods of CCPP in easy way, purposive and convenient through amplification and sequencing of the organism from clinically suggestive samples (Manso-Silvan et al., 2011) [29].

Lorenzon S, et al. (2008) [30] discovered the modern quantitative PCR (real time PCR) with high specificity and rapid, answered the question for the problem of traditional (qualitative PCR), during identification and quantification of Mccp strains. Furthermore, loop-mediated isothermal amplification (LAMP) was applied by He Y, et al. (2014) [31] in relieving the obstacles of diagnostic specificity and sensitivity.

As CCPP is complicated to identify from different respiratory diseases, Settypalli TB, et al. (2016) [32] developed more sensitive and specific technique which is called Multiplex PCR and solved the constraints of diagnosis. As a result, Mccp can cheaply distinguished from respiratory diseases such as pasteurellosis and Pestis des Pestitis Ruminants (PPR) (Yatoo et al., 2019) [33].

PCR identification of Mccp is highest in lung tissues, followed by pleural fluids and lowest in nasal discharges as described by figure 2. Differentiations of CCPP by using the diagnostic methods of PCR or sequencing have currently outdated other diagnostic methods due to its rapidity and reliability. But, more attention is mandatory during PCR tests to avoid contamination and require sophisticated laboratory or technical personnel (Saeed & Osman, 2018) [10].

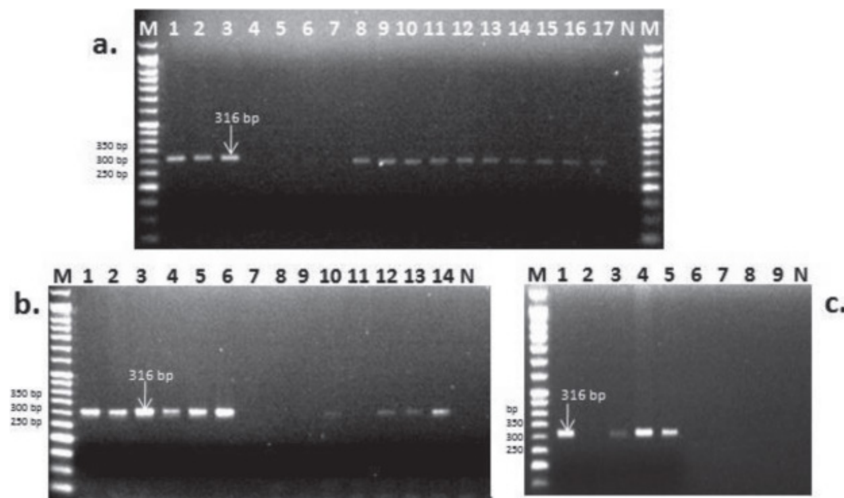

Figure 2: Gel electrophoresis of PCR products displaying a specific amplification of 316 bp of Mccp in, a) pleural; b) lung tissue and c) swab samples. Lane M, molecular weight marker (50 bp DNA ladder); numbered lanes are test samples; lane N, negative control.

In general, PCR technique is more sensitive diagnostic assay than cultural method and rated as the best and accurate method for confirmation of CCPP (Abraham et al., 2015) [34].

Newly, the best technique known sequencing was developed and opened a new era in research by identifying the organism at species stage from the specimen of infected animals such as pleural fluid on filter papers, even when it is in dry form. In addition, it is able to recognize strains precisely (the cleavage point to 16S rRNA and specifically the 'locus H2').

Control and prevention

The control and prevention of CCPP can be successful through mass vaccination of flocks and when movement of animals restricted (Teshome et al., 2021) [35].

In addition, CCPP is controlled by good sanitary measures such as; quarantine, disinfection and cleaning of areas where shoats are living. On another hand, early treatment of the disease by tylosine or tetracycline is important (OIE, 2014) [15].

CONCLUSION

As discussed in the current topic, the Confirmatory diagnosis is described as the isolation of Mccp from clinical samples of diseased lung. The diagnosis techniques such as; biochemical, immunological and molecular have been more important role in identification of CCPP isolates of infected animals. Serological tests to detect antibodies to Mccp includes CFT, LAT, and cELISA. The CFT is the most widely used serological test for CCPP. A cELISA is a suitable diagnostic technique for epidemiological investigations as it is recommended by different researchers. The PCR-based tests have been described as specific, sensitive and can be applied directly to clinical material, such as lung and pleural fluid. In general, present day PCR and sequencing has been used to set up molecular epidemiology and solving diagnostic challenges of CCPP. It was concluded that, postmortem examination, latex agglutination and PCR techniques, especially in specificity to confirm Diseases in outbreak and to help control rapidly. Hence, the refinement of seromolecular diagnostic techniques,

with special focus on convenience and field applicability, the good devising and skilled technical expertise, laboratory infrastructure and funding for proper diagnostic facilities in developing countries, including Ethiopia, with particular attention on both conventional diagnostics including culturing, biochemical, microbiological testing and advanced techniques such as: serological tests like CFT, ELISA, LAT and gene based techniques like; PCR, and sequencing are highly needed.

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