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A mini-review on diagnosis of contagious caprine pleuropneumonia

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ABSTRACT

Diagnosis of contagious caprine pleuropneumonia is imperative for timely detection and devising interventions that prevent disease spread and loss to farmers. Diagnosis of contagious caprine pleuropneumonia involves clinical signs, gross morphological lesions on postmortem, histopathology, culture and isolation, hematological, biochemical, serological and molecular diagnostic tests. Culture and isolation confirms the disease however it has been costly, cumbersome and difficult owing to the requirements of specific media, slow and difficult growth of causative agent *Mycoplasma capricolum* subsp. *capripneumoniae*. With the recent developments, diagnosis has comparatively eased by novel readymade media, advanced serological latex agglutination test (LAT), competitive enzyme linked immunosorbent assay (cELISA) or gene-based amplification of DNA, viz. polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), hybridization and sequencing than the cumbersome and lengthy conventional tests; however they have financial implications and require sophisticated laboratory infrastructure and technical manpower. The latex agglutination test (LAT) is rapid, simple, and better test for field and real-time diagnosis applicable to whole blood or serum and is more sensitive than the compliment fixation test (CFT) and easier than the cELISA. PCR and monoclonal antibody based ELISA being specific aid to confirmation of CCPP. Future thrust is on developing rapid, sensitive, and specific tests that are cheap and convenient for field application.

Keywords: Contagious caprine pleuropneumonia, Culture, Diagnosis, Enzyme linked immunosorbent assay, Mycoplasma, Polymerase chain reaction

Contagious caprine pleuropneumonia (CCPP) is a severe and highly contagious disease of small ruminants especially goats caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) characterized by respiratory manifestations (Yatoo *et al*. 2019, Ahaduzzaman 2020). It causes heavy morbidity (100%) and mortality (70–80%) (Liljander *et al*. 2019, Yatoo *et al.* 2019, Ahaduzzaman 2020, OIE 2021a). It has transboundary transmission and more than 40 countries are affected with the disease (Nicholas and Churchward 2011, Yatoo *et al.* 2019). Treatment, prevention and control of CCPP rely on its timely diagnosis (Thiaucourt *et al.* 1996, Yatoo *et al.* 2019). Diagnosis is confirmed mainly by culture and isolation while the serological and molecular tests aid in confirmation of diagnosis (Thiaucourt *et al*. 1996, Yatoo *et al*. 2019). These diagnostic tests are costly, time consuming and require well equipped laboratories and expertise (Thiaucourt *et al*. 1996, Liljander *et al*. 2015, Yatoo *et al*. 2019). However due to lack of infrastructure and cumbersome culture characteristics of Mccp including slow *in vitro* growth and contamination by other mycoplasma, field diagnosis is routinely based on clinical presentation of cases and postmortem examination of dead animals (Thiaucourt *et al*. 1996, Yatoo *et al.* 2019).

Serological tests like latex agglutination test (LAT) or slide agglutination test (SAT) are comparatively easy to perform, rapid, sensitive and does not require well equipped laboratory but are not specific especially SAT and may show cross reactivity (March *et al.* 2000, Kumar *et al.* 2016; Parray *et al*. 2019, Yatoo *et al*. 2019). CFT is recommended by OIE but detects antibody in initial stage and over short span of time (March *et al*. 2002, Mekuria *et al.* 2008). PCR and ELISA are specific but have financial implications and involve lengthy procedures and require sophisticated infrastructure and technical expertise (Liljander *et al*. 2015, Yatoo *et al*. 2018, 2019; OIE 2021b). However novel versions of these diagnostic tests are comparatively simpler and convenient to perform under ordinary settings

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(Liljander *et al.* 2015, Yatoo *et al*. 2018, 2019).

Thus considering the pros and cons of these available diagnostics, it is imperative to develop diagnostic tests that are accurate and specific for diagnosis of CCPP, rapid to perform, convenient in application, does not require high-end laboratory equipments, and are field applicable and easy to perform under field settings.

Currently diagnostic tests are being devised that are specific to CCPP, have rapid-point of time relevance and does not require well established laboratories. This review gives a brief account of the various diagnostic tests and developments in improvisation.

Common diagnostic tests for CCPP

General clinical diagnosis: Clinical diagnosis of CCPP is by clinical signs including respiratory distress, nasal discharge, coughing, sneezing, fever, thorax pain, anorexia, and weakness (El-Deeb *et al*. 2017, Tharwat *et al.* 2017, Yatoo *et al*. 2019). Postmortem lesions reveal fibrin deposition, pleural fluid exudation, marbling, congestion, hepatisation, and occasionally sequestra formation (El-Deeb *et al*. 2017, Elhassan and Salama 2018, Parray *et al.* 2019). Ultrasonography has been used as diagnostic tool for CCPP (Tharwat *et al.* 2017). Though these clinico-pathological signs are peculiar to CCPP but cannot be treated as confirmatory hence laboratory diagnosis is important.

Laboratory diagnosis: It involves culture and isolation, haemato-biochemical investigation, serological and molecular identification.

Culture and isolation: Culture and isolation is the confirmatory test for CCPP (Thiaucourt *et al*. 1996, Yatoo *et al.* 2019). Culture involves inoculating samples from affected animals into mycoplasma specific media. Samples from live animals include broncho-alveolar washings or pleural fluid obtained by puncture whereas lung lesions, lymph nodes, and pleural fluid are samples from dead animals (Thiaucourt *et al.* 1996, Yatoo *et al.* 2019, Ahmad *et al*. 2020, OIE 2021a,b). Sterile nasal swabs are also being used for sampling of nasal discharges. Pleuropneumonia-like organisms (PPLO) media that are specific media for mycoplasma are being used now-a-days for culture purposes (Yatoo *et al*. 2019, Baziki *et al.* 2020). Mccp requires selective media that are very rich and contain high percentage of serum (Yatoo *et al*. 2019, OIE 2021b). Fluid samples are directly cultured on broth media whereas solid tissue samples are first homogenized in buffer saline (Thiaucourt *et al*. 1996, OIE 2021b). Growth in broth takes 4-15 days in the form of turbidity but is usually faint. Comet formation may also be noted in undisturbed broth media (Thiaucourt *et al*. 1996, Yatoo *et al*. 2019, OIE 2021a,b). On agar typical small fried egg colonies of 0.1 to 0.5 mm are noted. Though culture of Mccp confirms CCPP however this is a lengthy and tedious process, requiring costly media and is complicated by slow growth of Mccp and frequent contamination by other mycoplasma. Nevertheless novel improved and readymade media have overcome some of these limitations. Further organisms isolated should be subjected to confirmatory molecular, biochemical or immunological methods.

Hematological tests: Hematological parameters have been evaluated in CCPP affected goats but have not shown any significant differences (Abdelsalam *et al*. 1988, Yatoo *et al.* 2019). Anaemia and leucocytosis have been reported in mycoplasma and CCPP affected goats (Mondal *et al*. 2004, Yatoo *et al.* 2019).

Biochemical tests: These tests are based on metabolic characteristics of the Mccp that can be explored for detection purposes. These include fermentation of glucose, digestion of casein and serum, and reduction of tetrazolium (Adehan *et al*. 2006, Soayfane *et al.* 2018, Yatoo *et al.* 2019). Hypoproteinaemia, rise in liver enzymes, calcium and glucose have been reported in mycoplasma affected goats (Mondal *et al*. 2004). Changes in acid-base balance, blood gases, and hematobiochemical profiles in blood and thoracic fluid of CCPP affected goats has been evaluated (Tharwat 2021). Blood PCO_2, PO_2, BE, HCO_3, TCO_2, and SO_2 have been found low in CCPP affected goats whereas AnGap has been found higher (Tharwat 2021). Further metabolic acidosis has been noted in CCPP affected goats (Tharwat 2021).

Serological methods: These tests involve detection of antigens of Mcpp or antibodies against these antigens. They include compliment fixation test (CFT) (MacOwan and Minette 1976) and enzyme linked immunosorbent assay (ELISA) that detects glycolipid and protein antigens or antibodies, respectively (Peyraud *et al*. 2014, OIE 2018), slide agglutination test (SAT) that detects nonspecific antibody against Mccp in serum (Litamoi *et al.* 1989) or latex agglutination test (LAT) that detects specific antibody against Mccp in serum (Rurangirwa *et al.* 1987, March *et al*. 2000, Yatoo *et al.* 2019). Others include indirect fluorescent antibody assay (IFAT), and passive or indirect haemagglutination test (IHT) (Muthomi and Rurangirwa 1983, OIE 2018).

A competitive enzyme-linked immunoassay (cELISA) for CCPP has been developed that is heat-stable laboratory diagnostic kit**.** It is suitable for prevalence and vaccine efficacy. It has limitations of not being able to detect acute outbreak of CCPP (Peyraud *et al.* 2014). Many antigens of Mccp that can serve as both vaccine candidates and diagnostic candidates have been reviewed by Yatoo *et al.* (2019). Baziki *et al.* (2019) have developed and evaluated epitope-blocking ELISA for detection of antibodies against Mccp in goat serum. This ELISA is 93% sensitive and 88% specific, and can help in detection of antibodies for the diagnosis CCPP and for sero-surveillance during vaccination campaigns (Baziki *et al.* 2019). An immuno-capture ELISA (ICE) has been developed by Baziki *et al*. (2021) for specific detection and quantification of the Mccp antigen in the CCPP vaccine. *In vitro* ICE method has shown correlation with an *in vivo* sero-conversion in goats and a sensitivity of 30 ng/ml (Baziki *et al.* 2021).

Molecular identification: Polymerase chain reaction (PCR) is the choice of diagnostic tests applicable directly

on clinical materials such as lung tissue and pleural fluid, being specific and overcoming difficulties of culture and isolation (Bölske *et al.* 1996, OIE 2018). Initial PCR assay for Mccp was developed by Bascunana *et al*. (1994) that amplifies 16S rRNA gene of the mycoides cluster. The amplified product is cleaved under restriction fragment length polymorphism (RFLP) by restriction endonuclease PST1 for identifying Mccp amplicon. Another PCR assay was devised by Woubit *et al*. (2004) using arcD gene as target for the specific amplification of a 316 bp-long DNA fragment. This gene is specific to Mccp. Lorenzon *et al.* (2008) utilized same primer sets as of Woubit *et al*. (2004) for quantitative PCR method. Manso-Silván *et al.* (2007) explored multilocus sequence typing for assigning a precise phylogenetic position to the mycoplasmas of the mycoides cluster. Liljander *et al*. (2015) developed a rapid, specific, and sensitive assay employing isothermal DNA amplification using recombinase polymerase amplification (RPA) for Mccp. The assay is cost effective, can be applied directly to samples, and has ease of operation under field settings. It can detect 5×10^3 and 5×10^4 cells/ml corresponding to genomic DNA and bacterial culture from *Mccp* strain ILRI181. Fluorscent signal is produced within 15–20 min using pleural fluid directly from affected animals without DNA extraction.

These gene or DNA-based tests have higher specificity and sensitivity, and less cost involvement. They also help in the differentiation of Mccp from other pathogens (Settypalli *et al*. 2016) and members of mycoides cluster (Taylor *et al.* 1992, Thiaucourt *et al*. 1992, Bascunana *et al.* 1994, Bolske *et al*. 1996, Hotzel *et al*. 1996, Bashiruddin 1998, Woubit *et al*. 2004, Lorenzon *et al*. 2008, OIE 2018). Besides PCR (Bascunana *et al*. 1994, Bolske *et al*. 1996, Hotzel *et al*. 1996, Woubit *et al.* 2004, Lorenzon *et al*. 2008, OIE 2018, Liljander *et al.* 2015), DNA probes (Taylor *et al.* 1992, Bonnet *et al.* 1993), hybridization (Maigre *et al*. 2008) and sequencing (Thiaucourt *et al.* 2000, Manso-Silván *et al.* 2011, Dupuy and Thiaucourt 2014, Falquet *et al*. 2014) are utilized for diagnosis of CCPP and identification of Mccp (Yatoo *et al*. 2019). Complete genome sequencing of *Mycoplasma capricolum* subsp. *capripneumoniae* Strain M1601 has been done by Chu *et al.* (2011) and genome-wide analysis of this sequenced Mccp strain M1601 has been done by Chen *et al.* (2017). These can be helpful in identification and understanding pathogenic mechanisms and genetics of Mccp (Chu *et al.* 2011, 2017). Sequencing has been used by Li *et al.* (2020) for comparative genomics analysis of *Mycoplasma capricolum* subsp. *capripneumoniae* strain 87001. A whole-genome worldwide molecular epidemiology approach for CCPP has been devised by Loire *et al*. (2020). This can serve as gold-standard for high-resolution typing procedure for the surveillance of contagious caprine pleuropneumonia (Loire *et al*. 2020).

Applications of diagnostic tests

These tests have been used in diagnosis of CCPP besides molecular characterization, evaluation of immune response, vaccine quality and standardization, pathogenesis and risk factors. This helps in timely detection, treatment, prevention and control of CCPP. Of the various diagnostics culture and isolation, SAT or LAT, cELISA and PCR remains the most commonly followed tests. Selim *et al.* (2021) and Parray *et al.* (2019) used a commercial cELISA kit (IDEXX, Montpellier, France) for studying seroprevalence and evaluating risk factors of CCPP. El-Deeb (2017), Abd-Elrahman *et al.* (2020) and Ahmad *et al*. (2020) used polymerase chain reaction assay for molecular identification of Mccp using protocol of Woubit *et al.* (2004) whereas Parray *et al*. (2019) used protocol of Bascunana *et al.* (1994). Elhassan and Salama (2018) used LAT and Parray *et al.* (2019) used SAT for studying seroprevalence of CCPP. Despite the routine diagnostics, novel approaches are being evaluated for exploring further aspects relating to CCPP in addition to diagnosis. Thiaucourt *et al*. (2018) used tandem mass spectrometry for evaluating quality of vaccines for CCPP. Zhang *et al.* (2021) used high-resolution melting curve analysis for rapid detection of *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma capricolum* subsp. *capripneumoniae*. Yatoo *et al.* (2019a,b) and Jores *et al.* (2020) have summarized necessity for future thrust areas including antigen evaluation, development of diagnostics and vaccines.

Conclusion

Diagnosis of CCPP is essential for treatment, prevention and control. Though culture and isolation is hectic, laborious and time consuming but is confirmatory test for Mccp. Biochemical tests only support the diagnosis and are not confirmatory. Evolution of serological tests from nonspecific indirect haemagglutination test, compliment fixation test, slide agglutination test to specific latex agglutination test improved diagnosis but has limitations of cross reactions which are comparatively overcome by immunoassays including the recent competitive ELISA. However these diagnostics are costly; require well established laboratory settings and technical expertise. Nevertheless identification of Mccp by PCR methods has become the routine protocol and facilitates confirmatory diagnosis with novel protocols being specific, convenient and applicable even under field conditions under ordinary settings. Future thrust should be on the field applicable diagnostic tests that besides being highly specific and sensitive need to be cheap, easy to perform and does not require sophisticated laboratory infrastructure and technical manpower.

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