A mini-review on diagnosis of contagious caprine pleuropneumonia

IZHAR UL HAQ RATHER1, OVEAS RAFFIQ PARRAY1, QURAT UL AIN1, RIYAZ AHMED BHAH1, MUHEET1, SHARJEEL WAN11, MOHSINA MUSHTAQ1, JALLAL UDIN PARRAH1, AMATUL MUHEEH1, KULDEEP DHAMA2, YASHPAL SINGH MALIK3 and MOHD IQBAL YATOO1,2,3

Sher-e-Kashmir University of Agricultural Sciences and Technology–Kashmir, Shalimar, Jammu and Kashmir 190 025 India

Received: 28 August 2021; Accepted: 15 September 2021

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 readyemade 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).

Present address: 1Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, Sher-e-Kashmir University of Agricultural Sciences and Technology–Kashmir, Jammu and Kashmir. 2Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh. 3College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab. 4-5Corresponding author e-mail: iqbal_yatoo@gmail.com

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.
developments in improvisation. It gives a brief account of the various diagnostic tests and does not require well established laboratories. This review is specific to CCPP, have rapid-point of time relevance and 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 PCO2, PO2, BE, HCO3, TCO2, and SO2 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 Mccp or antibodies against these antigens. They include complement 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. 2019), and haemagglutination test (IHT) (Muthomi and Rurangirwa 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 immunocapture 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 mycoplasmas 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 mycoplasmic 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 $5 \times 10^3$ and $5 \times 10^4$ cells/ml corresponding to genomic DNA and bacterial culture from Mccp strain ILRI181. Fluorescent 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 mycoplasmas 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 (Maire 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. 2019a,b). 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.

ACKNOWLEDGEMENTS

Authors are thankful to SKUAST-K authorities for support. Funding from Science and Engineering Research Board (SERB) and Science for Equity Empowerment and Development (SEED)-Department of Science and Technology (DST), Government of India is highly acknowledged.
REFERENCES


Manso-Silván L, Dupuy V, Chu Y and Thiaucourt F. 2011. Multilocus sequence analysis of *Mycoplasma capricolum* subsp. *capripneumoniae* for the molecular epidemiology of contagious caprine pleuropneumonia. *Veterinary Research*
42(1): 86.