Current Knowledge on Peste des petits ruminants: A comprehensive review on clinical signs, diagnostic test and vaccination

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Abstract
Peste des petits ruminants (PPR) is currently considered as one of the main transboundary animal diseases that constitutes a threat to small ruminant production in many developing countries. It is a viral disease of sheep, goats and wild ruminants (Impala, Gazelles, Springbuck), with goats being more severely affected. The disease is caused by a Morbillivirus, peste des petits ruminants virus (PPRV) that belongs to the family Paramyxoviridae. The disease cause high mortality and morbidities to the tune of 90 to100% in endemic areas with high economic impact. It was estimated in the FAO-OIE Global eradication strategy report that the direct annual losses due to PPR are between USD 1.2 and 1.7 billion. This review is an attempt to summarize the recent advancement in PPRV specific clinical signs, diagnostic tests, vaccination and disease control strategies. Clinically, the disease is characterized by sudden dullness and fever, depression, anorexia, cutaneous nodules, severe purulent ocular and nasal discharges resulting in reddening of conjunctiva and matting of the eyelids, respiratory distress and coughing. The pathology caused by PPR is dominated by necrotizing and ulcerative lesions in the mouth and the gastro-intestinal tract. The recent molecular characterization of PPRV virus isolates subdivides them into four genetically distinct lineages (I, II, III and IV). Both viral isolation, molecular and serological tests are reliable tests to confirm presence of the disease. The virus is transmitted through a close contact with an infected animal, although oral transmission is possible through ingestion of contaminated feed and water. Separation of infected animals from healthy animal at the first stage can minimize the chance of transmission of the virus from infected to healthy animals. Advanced molecular techniques are more accurate to detect the timely diagnosis of the disease. Moreover, the vaccination campaign will improve the prevention of the disease.

Keywords: Peste des petits ruminants, symptoms, diagnostics tools, vaccines, control

Introduction

Peste-des-petits-ruminants (PPR) is a transboundary animal viral disease of small ruminants that largely affect goats and sheep, but can also infect and cause disease in wild species (springbuck, gazelles and impala) as well as in camels (Banyard et al., 2010; Sarker and Islam, 2011; Muse et al., 2012). PPR is an epidemic in Sub-Saharan Africa, the Middle East and Asia and it is caused by an enveloped, non-segmented single-strand negative-sense RNA virus of 15,948 nucleotides which six structural (Nucleoprotein, phosphoproteins, matrix proteins fusion protein, hemagglutinin proteins and polymerase proteins) and two non-structured (C and V) proteins (Housawi et al., 2004; Swa et al., 2009; Qin et al., 2012; Naveen et al., 2014). Although PPR was first reported in Africa in the 1942, the number of countries in Africa reporting PPR outbreaks to the African Union-Inter-African Bureau for Animal Resources (AU-IBAR, 2013) has increased to over 20 between 1988 and 2009 (Parida et al., 2016). Based on partial sequence analysis of fusion and nucleoprotein, the recent molecular characterization of PPRV virus isolates divide them into four genetically distinct lineages (I, II, III and IV) (Esmaeilzad et al., 2011; Luaka et al., 2011; Sharawi and Abd-El-Rahim, 2011). Lineage I represented mainly in Western African isolates from the 1970s and recent isolates from Central Africa; lineage II by West African isolates from the Ivory Coast, Guinea and Burkina Faso; lineage III by isolates from Eastern Africa and the Sudan; lineage IV includes viruses isolated from recent outbreaks across the Arabian Peninsula, the Middle East, southern Asia and recently across several African territories (Banyard et al., 2010; Parida et al., 2016). The classification of PPRV into lineages has broadened the better understanding of the molecular epidemiology and worldwide movement of PPR viruses (Balamurugan et al., 2014). Lineage IV has been distinct to Asia even though it has been reported in west, north, central east and southern Africa thereby becoming the most widely circulating lineage (Banyard et al., 2010; Kwiatek et al., 2011).

It was estimated in the FAO-OIE Global eradication strategy report that the direct annual losses due to PPR are between USD 1.2 and 1.7 billion, the estimated current expenditure on PPR vaccination ranges between USD 270 and 380 million and annual impact of PPR alone valued at between USD 1.45 and 2.1 billion per year. Currently, around 70 countries have reported infection to the OIE and another 50 are considered at risk for PPR. Out of these infected countries, more than 60% are in Africa (OIE, 2008). The emergence of the PPRV virus required a proper diagnostic and control strategies in order to contribute to the reduction of mortality and morbidity rates due to PPR, therefore, this review paper aimed at highlighting the PPR diagnostics tests, clinical signs and control strategies.

Symptoms of PPR

The onset of the disease is marked by sudden dullness and fever with high body temperature between 40°C and 41°C. Other characteristics of the disease include depression, anorexia, severe purulent ocular discharges, resulting in reddening of conjunctiva and matting of the eyelids, severe purulent nasal discharges, respiratory distress and coughing (Roeder and Obi, 1999; Diallo et al., 2007). Kumar et al. (2004) and Chauhan et al. (2009) reported erosions on the mucous membrane of the buccal cavity accompanied by marked salivation with ulcers developing in the mucosa of the alimentary, respiratory and urinary tracts. Diarrhea is accompanied or preceded by a sudden drop in core body temperature followed by death, in fatal cases, five to 12 days after the onset of disease (Baron et al., 2011). Abdalla et al. (2012) added that pregnant animals with PPR clinical signs may abort with morbidity and mortality rate. However, these rates are higher in young animals than in adults (Baron et al., 2011). Hard non-painful nodules all over the body have also been noted by Baron et al. (2011). A clear watery discharge starts to flow from month, eyes and nose, later becoming thick and yellow as a result of bacterial secondary infection. Serous to mucopurulent nasal discharge crust over and occlude the nostrils causing sneezing and difficulty in breathing (Fig. 1) as describe by Roeder and Obi (1999). Kihu et al. (2012) found serous mucopurulent ocular discharges ensued matting together of the eyelids (Fig. 2). One to two days after fever has set in, the mucous membranes of the mouth and eyes become much reddened. The epithelial necrosis causes small pin-point greyish areas on the gums, dental pad, palate, lips, inner aspects of the cheeks and upper surface of the tongue (Fig. 3) as described by Berhe (2006). These areas increase in number and size and join together. The lining of the mouth is changed in appearance. It becomes pale and coated with dying cells and, in some cases; the normal membrane may be completely obscured by a thick cheesy material. Underneath the dead surface cells, there are shallow erosions. Kgotele et al. (2014) found the periorbital edema and cutaneous nodules in PPR infected goat in Tanzania (Figure 4).

Pathology of the disease

The PPR affected animal carcass is usually emaciated, the hindquarters soiled with soft/watery faeces and the eyeballs sunken. The pathology caused by PPR is dominated by necrotizing and ulcerative lesions in the mouth and the gastro-intestinal tract (Roeder et al., 1994). Abomasum is congested with
Fig. 1: Mucopurulent nasal discharge and swollen upper lips (Roeder and Obi, 1999).

Fig. 2: Mucopurulent ocular discharge matting hair from canthus of the eye (Kihu et al., 2012)

Fig. 3: Erosive stomatitis with dead cells on the gums involving the inside of the lower lip (Berhe, 2006).

Fig. 4: periorbital edema and cutaneous nodules (Kgotlele et al., 2014).

lining hemorrhages. The rumen reticulum and omasum rarely exhibit lesions. Occasionally, there may be erosions on the pillars of the rumen. The omasum is a common site of regularly outlined erosions often with oozing blood. Lesions in the small intestine are generally moderate, being limited to small streaks of hemorrhages and, occasionally, erosions in the first portions of the duodenum and the terminal ileum. The large intestine is usually more severely affected, with congestion around the ileo-cecal valve, at the ceco-colic junction and in the rectum. The circle shows congested mesenteric lymph nodes while the arrows show congested mediastinal lymph nodes of PPR-suspected animals. Kgotlele et al. (2014) found congested lymph nodes in the gastrointestinal and in respiratory system, pneumonia and hemorrhage draining internal organs in a Tanzanian goat suspected with PPR after postmortem (Fig. 5).

Moreover, Roeder and Obi (1999) and Truong et al. (2014) found in the posterior part of the colon and the rectum, discontinuous streaks of congestion “zebra stripes” form on the crests of the mucosal folds. The lung is dark red or purple with areas firm to the touch, mainly in the anterior and cardiac lobes show evidence of pneumonia (Fig. 6).

**Histology of the disease**

_Peste des petits ruminants’_ virus causes epithelial necrosis of the mucosa of the alimentary and respiratory tracts marked by the eosinophilic intracytoplasmic presence and intra nuclear inclusion bodies (Brown et al., 1991). Multinucleated giant cells (syncyitia) can be observed in all affected epithelia and in the lymph nodes where there is severe depletion of lymphocytes (Truong et al., 2014). Munir et al. (2013) showed that in the lungs multifocal degeneration, ulceration and necrosis, followed by alveolar type II pneumocytes hyperplasia, which mostly ends up with syncytial cell formation is a prominent feature. Lymphocytes, plasma
cells and histocytes penetration into the alveolar septae leads to its hypertrophy and desquamation with alveolar casts (Munir et al., 2013). Later on, Troung et al. (2014) found that the intestinal lesions are characterized by blunt villi, degeneration of surface and crypt epithelial cells; lamina propria expansion by a primarily mononuclear infiltration with scattered syncytial cells.

**Diagnostic of Peste des petits ruminants**

Although clinical signs and gross pathological findings may be typical of PPRV infection, alone they cannot be used to confirm PPR. This is because respiratory diseases in small ruminants have multiple causes and as a result the disease may go undetected or misdiagnosed as it spreads especially by animals that show no overt clinical signs. PPRV diagnostic may be performed through the isolation of the virus, detection of viral antigens, nucleic acid isolation (viral RNA), sequencing (Sanger or Next Generation Sequencing) and detection of specific antibody in the serum (Gopilo, 2005).

**Virus isolation**

This technic consists to isolate the PPR virus in cultured cells. Roeder and Obi (1999) showed that this method of diagnosis can provide live virus for biological characterization studies and the isolated viruses are stored for later studies. However, the samples including heparinized blood, eye and nasal swabs (from live animals), tonsil, mesenteric lymph nodes, spleen, section of colon and lung from necropsied cases give better chance for virus isolation because it was proved that PPR virus circulating in big amount in these liquids. Therefore, for successful isolation, samples must be collected during the hyperthermic phase and submitted to the testing laboratory in cold ice or in RNA later. The most widely used cell culture systems are primary lamb kidney and ovine skin and Vero cells (Adombi et al., 2011).

**Molecular techniques**

**Nucleic acid recognition methods**

The rapid method for molecular diagnostic is the reverse transcription polymerase chain reaction (RT-PCR) technique based on the amplification of whole or parts of any part of the virus genome. However, the N and F protein genes have been more developed for the specific diagnosis of PPR (Couacy-Hymann et al., 2002). This technique is 1000 times more sensitive than classical virus titration on Vero cells with the advantage that results are obtained in 5 hours, including the RNA extraction, instead of 10–12 days for virus isolation. Moreover, this technique is very specific because it targets the gene of your interest in the genome using the specific primers. This technique can be complement by a better and more sensitive technique which is transcriptase polymerase chain reaction or quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) (Kwiatek et al., 2010; Li et al., 2010).

Another molecular technique is the reverse transcription-loop mediated isothermal amplification (RT-LAMP). This assay has an intrinsic potential for point of care diagnosis focused on the genetic detection of causative PPR virus (PPRV) in field conditions. This method is consisted of precipitation of the sample buffer out of virus envelope and capsid proteins through ammonium sulphate precipitation and exposes viral RNA present in the clinical sample to the LAMP reaction mixture (Ashraf et al., 2017). LAMP based amplification of target nucleic acid is based on isothermal amplification of template DNA utilizing the strand displacement activity of Bst or Bsm DNA polymerase enzyme originated from *Bacillus stearothermophilus* or *Bacillus smithii*, respectively (Nagamine et al., 2002).

**Specific cDNA hybridization**

This method is used for nucleic acid technology to the detection of the eradicated rinderpest (RP) and *peste...*
des petits ruminants (PPR) viruses by using cDNA probes corresponding to the nucleocapsid gene of each virus and labelled with isotope [P³²] nucleotides. This hybridization technique is used to clearly identify the virus involved in an outbreak (Taylor et al., 1990). Unfortunately, this hybridization cannot be used widely because it requires fresh specimens and in addition to the short half-life of [P³²] isotope, and there is constraints with the handling of isotopes due to their reactivity. Diallo et al. (1989) described that cDNA probes was a rapid method for differential diagnosis of infections caused by RPV or PPRV, derived from the mRNAs for the nucleocapsid protein of each virus, which can be used to distinguish unequivocally the two virus types rapidly.

Serological tests

Haemagglutination Test (HA)

The haemagglutination test is one of the cheapest, easiest and effective methods for PPRV diagnosis that has advantage of the differentiating PPR from RPV. Osman et al. (2008) used the HA method for detection of PPRV antigen in Forty lymph nodes and spleen samples from suspected cases of PPR in both sheep and goats. The HA test was carried out using goat, chicken and pig RBCs.

Immunofluorescent Antibody Test (IFAT)

The IFAT is also one of the simple and quick methods for PPRV antigen for diagnostic. It has the advantage that facilities are available in most veterinary laboratories. The IFAT technique is reported to have 100% specificity in detection of PPR antigen in conjunctival smears from suspected PPR cases found from a field outbreak (Sumption et al., 1998). Moreover, the immunofluorescence antibody test (IFAT) is also frequently used as a reference test in validation of new diagnostic methods and estimating the canine leishmaniasis (CanL) true prevalence in the Mediterranean basin (Adel et al., 2016).

Agar Gel Immunodiffusion Test

This method detects virus antigens by the agar gel immunodiffusion test (AGID). It is also simple to handle, fast and cheap process. One disadvantage of this test is that it does not discriminate PPR and RP viruses, therefore, further tests are needed. Using this test, results can be obtained in one day, but the test is not sensitive enough to detect mild forms of PPR due to the low excreted quantity of viral antigen (Kumar et al., 2004; Balamurugan et al., 2014).

Counter immunoelectrophoresis

The method of counter immunoelectrophoresis (CIEP) works almost in the same principle as the AGID except that the gel has an electric charge to improve the test sensitivity. Counter immunoelectrophoresis is one of the most rapid tests for detecting viral antigen. It has been shown that both CIEP and AGID are group-specific and cannot differentiate PPR and RP infections (Osman et al., 2008). Balamurugan et al. (2014) also showed that the CIEP was comparatively more sensitive and rapid method than that of AGPT, but could not differentiate between RPV and PPRV infection.

Immunoperoxidase Staining (IP)
/Immunohistochemistry (IHC)

This method involves the combination of histopathology with immunohistochemical staining (immuno peroxidase). It is a useful test performed on formalin-fixed material and can discriminate between RP and PPR when performed with specific monoclonal antibodies. The IHC reaction is characterized by the presence of light to dark brown, fine to coarse granular area in cells and tissues (Kumar et al., 2004). Chen et al. (2010) confirmed that IHC is one of the useful research tool used to localize specific antigens in tissue sections with labelled antibodies based on antigen-antibody interactions. The marker including fluorescent dye, enzyme in general, radioactive element or colloidal gold can be used for immune reactive products visualization.

Immunocapture ELISA (IC-ELISA)

The immunocapture ELISA (ICE) method can detect rapidly and sensitively the viral antigens. Moreover, this test can differentiate between PPR and rinderpest. Considering the similarly in geographic distribution of the two diseases and knowing that they can affect the same animal, this method seems to be of a great importance (Diallo et al., 2007).

Sandwich ELISA (S-ELISA)

PPR virus-specific neutralizing monoclonal antibodies are used in a simple and rapid double-antibody Sandwich ELISA for specific detection of PPRV antigen in goat/sheep tissues and secretions. Singh et al. (2004) described a sandwich ELISA test using PPR specific monoclonal antibody (clone 4G6) to an epitope of nucleocapsid protein. This technique is also known to be simple, rapid, cost effective and convenient for intensive clinical surveillance and routine diagnosis of the disease. The results from a study on comparative evaluation of RT-PCR with sandwich-ELISA for detection of Peste des petits ruminant in sheep and goats in India showed a low sensitivity and specificity by F-gene based RT-PCR when compared with sandwich ELISA suggesting that some other highly sensitive and specific primers should be explored for detection of PPR by RT-PCR (Mahajan et al., 2013).
**Enzyme-Linked Immunosorbent Assay (ELISA)**

Conventional serological tests such as haemagglutination test, enzyme linked immunosorbent assay (ELISA) and virus neutralization assay often fail to differentiate PPR from rinderpest. An alternative system for sero-surveillance and sero-monitoring of PPR is a monoclonal antibody based competitive-ELISA (Singh et al., 2004). The cELISA test is based on the competition between the anti-PPR monoclonal antibody which is directed against the haemagglutinin protein of PPR virus and the antibodies in the serum sample. Competitive Enzyme-Linked Immunosorbent Assay sensitivity is 99.4% and specificity 94.5% (Singh et al., 2004). The presence of antibodies to PPR virus in the serum samples blocks reactivity of the monoclonal antibodies, which causes reduction in the expected color following the addition of enzyme labelled anti-mouse conjugate and chromogen solution.

The blocking ELISA (B-ELISA) is proved to be sensitive, specific, simple and more rapid for detection of PPR antibodies, but unfortunately the VNT, B-ELISA may be affected by the contamination and the quality of sera (Luka et al., 2011; Abdalla et al., 2012).

**Haemagglutination Inhibition Test (HI)**

This technique consist to absorb out the cross reacting antibodies to rinderpest antigen from a PPR serum and leaving the specific antibody to PPR, which is determined by haemagglutination-inhibition test. The HI test is cheaper, simple and yet reliable. However, there is a need to standardize the commonly available PPR antigen for easy evaluation of efficacy of PPR vaccination efforts (Naveen et al., 2014).

**Counter Immunoelectrophoresis (CIEP)**

The CIEP is one of the highly adaptable methods for serum antibody titration but can also be used for sero-epidemiological and experimental surveys to diagnose PPR. I was previously confirmed that its rapidity, simplicity and sensitivity made it a suitable technique in serological studies of PPR (Osman et al., 2008; Mahajan et al., 2013).

**Agar Gel Diffusion Test (AGDT)**

The AGDT test is used to detect the antibodies against PPR in the sera of the affected goats or sheep. This method is described to the passive diffusion of soluble antigens and/or antibodies towards each other leading to their precipitation in a gel matrix. It is also called ouchterlony test, double immunodiffusion test or agar gel precipitin (AGP). Osman et al. (2008) confirmed the useful of this test for diagnosis of PPR in the field.

**Virus Neutralisation Test (VNT)**

The virus neutralization test (VNT) is sensitive and specific, but more expensive and time-consuming. This test is the oldest most reliable test for detection of morbillivirus antibodies. Serum against either PPR or RP may neutralize both viruses, but would neutralize the heterologous virus at a lower titer compare to the homologous virus. Therefore, for differentiation purpose reciprocal cross neutralization is used (Taylor et al., 1990). Hu et al. (2012) in a study on rescue of recombinant PPR virus found that the recombinant virus allowed more rapid and higher throughput assessment of PPRV neutralization antibody titer via the virus neutralization test (VNT) compared with the traditional method.

**Precipitinogen Inhibition Test (PIT)**

The success of PIT test which is one of the oldest serological tests is basically based on the ability of antibody in serum to inhibit diffusible virus antigen (precipitinogen) from developing a precipitin line against hyper immune serum in AGPT. Durojaiye (1987) observed that the PIT test is more sensitive (33%) as compared to Neaiurisation Test (NT) (28%).

**Vaccine and vaccination of the disease**

In the past, vaccination with the rinderpest vaccine was used to control PPR because of the existence of a strong antigenic relationship between PPR and rinderpest viruses (OIE, 2008; Luka et al., 2011). Currently, PPRV homologous vaccine made from strain Nigeria PPRV 75/1LK6 Vero 70 is in use in control of PPR in endemic areas. New PPR recombinant marker vaccines are under development, they will enable differentiation between infected and vaccinated animals for sero-surveillance and sero-monitoring purposes (Baron et al., 2011; Balamurugan et al., 2014). Strong support of diagnostics and timely vaccination of the susceptible population based on an understanding of the epidemiology of the disease may help eradicate PPR as it was done with rinderpest. Different type of PPR vaccines including conventional, thermostable, recombinant and edible vaccines has been developed and used from control/eradication of said disease world widely (Abubakar et al., 2012). However, a differentiation of infected from vaccinated animals (DIVA) vaccine/test would improve epidemiological data by tracking of infection in areas where there has been partial vaccination. Efforts are being made to develop thermoressistant vaccine and PPR recombinant marker vaccines (Berhe, 2006). The recombinant marker vaccines will make it possible to differentiate infected and vaccinated animals for sero-surveillance and sero-monitoring purposes while thermoressistant vaccine will reduce the cost of vaccination by side-stepping the cold chain storage.

**Control and prevention of the disease**

Different control and preventive strategies can be used for surveillance or eradication of PPR in animal.
Separation of infected animals from healthy animal at the first stage can minimize the chance of transmission of the virus from infected to healthy animals. At the second stage, slaughter of apparent diseased and seropositive animals, proper dispose of all infected material and decontamination of items of infected sheep/goat flock are crucial for control/eradication of PPR (Diallo et al., 1989; Diallo et al., 2007). However, vaccination of animals seems to be a good option to minimize the risk of occurrence in any healthy animal flocks. In worldwide different immunization strategies against PPR has been used such as attenuated tissue culture rinderpest vaccine (TCRV), earlier immunization of small ruminants with lymph node and spleen materials containing virulent virus inactivated with 0.5-5 % chloroform, but now PPR homologous vaccine is available. Proper disposal of carcass and contact formites, decontamination and restriction on importation of sheep and goats from affected areas may help also to control PPR (Chauhan et al., 2009).

Conclusion

After the successful eradication of rinderpest from the globe, FAO-OIE has launched a progressive control program of PPR by replicating the tools and experience used in the rinderpest eradication process. It appears from this study that there is no clear differentiation in PPR specific clinical signs that can be used to confirm the presence of the disease. Laboratory based tests including virus isolation, molecular and serological tests have high sensitivity and specificity. Vaccination of animals seems to be a good option to minimize the risk of occurrence in healthy animals.

References

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