



Diagnosis and Control of Peste des Petits Ruminants Disease in Small Ruminants: A Review

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ABSTRACT

Peste des Petits Ruminants (PPR) is an acute highly contagious febrile disease of sheep and goats characterized by erosive and necrotizing stomatitis and associated with severe pneumo-enteritis and bronchopneumonia ended by recovery or death. The aim of the present study was to throw light on the diagnosis and control of PPR. Diagnosis of PPR depends on clinical signs, pathological lesions, and specific detection of the viral antigen, viral genome, or specific antibodies by serological tests and nucleic acid-based assays. The most commonly used diagnostic techniques are cell culture isolation, agar gel immunodiffusion, hemagglutination tests, immunocapture ELISA, and competitive ELISA. In addition to the abovementioned techniques, virus neutralization tests and reverse transcriptase PCR are used. Peste des Petits Ruminants is characterized by high fever associated with watery nasal and ocular discharges, mucopurulent stomatitis, and broncho-pneumonia. Moreover, severe bloody diarrhea and the disease associated with high levels of mortality reached up to 90%. The diagnosis of viral diseases is important in determining the control strategies. Therefore, it can be concluded that recent diagnostic tools are urgently needed not only for the diagnosis but also for following-up combating programs and control of viral diseases. Early and rapid complete identification of infectious viral agents in small ruminants as well as in the surrounding environment is recommended for effective control of PPR. The control program depends mainly on vaccination, hygiene and sanitation measures, and effective quarantine measures.

Keywords: Control, Dairy Ruminants, Diagnosis, Goat, Peste des Petits, Sheep

INTRODUCTION

Sheep and goats are susceptible to infection by many viruses. Viral diseases affect the productivity of both species and sometimes induce severe losses which appear in the form of high mortalities in the newborns, low growth rates, loss in body gain in adult animals in addition to early embryonic deaths, newborn mortalities, abortions, abnormal weak lambs and persistently infected animals (Onono et al., 2013). The clinical signs of viral diseases differ according to the type of the disease. Peste des Petits Ruminants (PPR) is an acute highly contagious febrile disease of sheep and goats characterized by erosive and necrotizing stomatitis and associated with severe pneumo-enteritis and bronchopneumonia ended by recovery or death (Ismail et al., 1995). The PPR is reported in most African countries, south of the Sahara and north of the equator, and in nearly all Middle Eastern countries and South-West Asia (OIE and FAO, 2015). The PPR virus (PPRV) is related to the family *Paramyxoviridae* that consists of two subfamilies, *Paramyxovirinae* and *Pneumovirinae*. Large, negative-strand RNA viruses characterize this family. The two subfamilies include several genera with important human and veterinary pathogens that cause diseases, such as canine distemper and rinderpest (RP). Although PPRV is famous as one serotype, the partial sequencing of the fusion protein (F) gene reveals the existence of 4 lines (I, II, III, and IV) (Banyard et al., 2010; Kardjadj et al., 2015). Commonly, the first two lineages II and I are existing in West Africa, while lineage III is present in East Africa and the Middle East, and lineage IV is present in Asia (Wasim et al., 2015). The first case of PPR in Egypt was recorded in 1988 by Ikram et al. (1988) in goats located in Giza Governorate. The disease is responsible for high rates of morbidity and mortality, leading to severe economic losses (El-Allawy et al., 1993). The disease is considered endemic in Egypt and has a seasonal occurrence in winter in most of the country (Fayed et al., 1994). Mouaz et al. (1995) reported an outbreak of PPR affecting sheep in 1994 in Giza province. The isolated virus was designated as Giza-94 after confirmation by ELISA and VNT (Abd El-Rahim et al., 2010). A recent study applied competitive ELISA on sheep and goat samples in two Egyptian governorates and recorded the prevalence of PPR antibodies. Giza governorate showed positivity of 63.8% in sheep and 45.7% in goats. Moreover, 71.7% of sheep and 45% of goats in the Beni-Suef governorate were positive (Mahmoud et al., 2017). Diagnosis of PPR depends on clinical signs, epidemiology, pathological lesions, and specific detection of the viral antigen, viral genome, or the specific antibodies in the tested samples by different serological tests and nucleic acid-based assays (Balamurugan et al., 2014; Kinimi et al., 2020). The most commonly used diagnostic techniques are culture

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isolation, agar gel immunodiffusion (AGID), hemagglutination assay (HA), immunocapture enzyme-linked immunosorbent assay (IC-ELISA), and competitive ELISA. In addition to the previously mentioned techniques, virus neutralization test (VNT) and reverse transcriptase polymerase chain reaction (RT-PCR) are used (Forsyth and Barrett 1995; Anderson et al., 2006). Infectious viral diseases in small ruminants such as PPR are difficult to control because of the insufficient data of the epidemiology, diagnosis and different control strategies in the herds where the problems are occurring. Infectious viral diseases of small ruminants hinder the expected benefits from these animals (Mahmoud et al., 2017). The diagnosis of viral diseases is important in determining of the control strategies. Therefore, the aim of this study was to throw light on the diagnosis and control of PPR in sheep and goats.

DIAGNOSIS OF PESTE DES PETITS RUMINANTS DISEASE

Sheep and goat susceptibility

The disease attacks primarily goats and sheep. Goats are more liable than sheep and show heavy losses. Goat breeds have different susceptibilities to PPR. Guinean breeds show more susceptibility than Sahelian breeds. The European breeds are readily susceptible. The age of the animal has also a significant effect, where lambs are highly susceptible than adults or unweaned young animals (Kardjadj et al., 2015). Sheep or goats aged 3–4 months are more susceptible to PPRV infection due to the diminishing the maternal antibodies (Burezq et al., 2020).

Clinical signs

The disease is characterized by a high fever reach to 40°C, associated with watery nasal and ocular discharges which later change to mucopurulent, stomatitis, and bronchopneumonia (Balamurugan et al., 2014). Moreover, severe diarrhea with an offensive odor has also been reported which may be bloody and often lead to death (Farougou and Gagara, 2013). This disease is characterized by high levels of morbidity and mortality up to 90% (Kwiatk et al., 2011). Recent researchers recorded deviations in the distribution and the varieties of clinical signs of the disease, represented in the emergence of PPRV lineage IV in northeastern and northern Africa (Albina et al., 2013; Kardjadj, 2018).

Post-mortem findings

The post-mortem findings include intensive red areas and congestion in the lungs with pneumonic changes. Large intestines and colon show hemorrhages (Zebra markings). Besides, enlargement of the spleen and lymph nodes in association with abomasal erosion has been reported by Abubakar et al. (2011) and Abubakar et al. (2015).

Differential diagnosis

Differential diagnosis must be applied to differentiate the other diseases that can be misdiagnosed with PPR and these include; signs of lameness differentiate Foot and Mouth Disease (FMD), there is no diarrhea in bluetongue disease, pock lesions differentiate capripox viruses, and necrotic stomatitis is not found in contagious ecthyma. Pneumonic pasteurellosis and caprine pleuropneumonia are characterized by respiratory illness alone. Although some PPR-infected cases do not show all the clinical signs, so differential diagnoses in these cases cannot be applied. As a result, laboratory confirmation of PPR is recommended for the definitive diagnosis (Radostits et al., 2006).

Virus identification

Virus isolation

Virus isolation represents the gold standard method for the diagnosis of PPR. Different types of cell cultures like Vero cells (African green monkey kidney cells) were used for PPRV isolation (Durojaiye, 1987). The characteristic specific cytopathic effect is in the form of cell rounding followed by grape-like formation, vacuolation, cytoplasmic granulation, and syncytia formation that appears after 3–5 days of infection. Virus isolation is not applicable in routine diagnostic assays as it is slow and needs special facilities and lacks the same sensitivity as RT-PCR (Santhamani et al., 2016).

Agar gel immunodiffusion

The comparative efficiency of competitive ELISA and the standard AGID for the diagnosis of PPR was inspected and the results showed that competitive ELISA has high sensitivity and specificity than AGID and could be used as a standard test, especially for the testing of the improperly kept samples. Some common serological tests like AGID and indirect ELISA were effective in the past for the primary diagnosis of PPR but there is a cross-reaction between PPR and RP bodies (Santhamani et al., 2016).

Sandwich ELISA

Sandwich ELISA (*s-ELISA*) is highly sensitive (99.8%) and specific (90.5%), and it is used for the detection of PPRV in the nasal and ocular secretions of infected sheep with PPR PCR (Mahajan et al., 2013; Santhamani et al., 2016; Mahmoud and Galbat, 2017).

Nucleic acid recognition methods

Most of the serological tests are slow, labor exhaustive, and of low sensitivity. Therefore, they are of low value in the primary diagnosis, but of high value in confirmatory steps and in epidemiological studies. Real-time RT-PCR and loop-mediated isothermal amplification (LAMP) assays are used for their quickness and high sensitivity in the detection of PPRV RNA from clinical samples to overcome the problems associated with the serological tests (Santhamani et al., 2016).

Multiplex PCR

The conventional RT-PCR is laborious and unaffordable for routine clinical diagnosis in poorly financed laboratories, especially in large sample numbers. Moreover, this technique is sensitive to cross-contamination during sample collection and processing which may lead to false positives (Santhamani et al., 2016). False negatives may occur due to unexpected alteration in the primer binding sites, destruction of the RNA, in addition to the existence of PCR inhibitors in the samples. To overcome the false negatives results, multiplex PCR must be applied in the presence of multiple primer pairs to amplify not only different but also specific regions of genes. There are two types of multiplex RT-PCRs. Two-step and single-step procedures are directed to the N and M genes to differentiate PPRV from the rinderpest virus (Balamurugan et al., 2006). The multiplex RT-PCR has higher sensitivity for PPR diagnosis than s-ELISA. Cross-contamination is reduced in single-step assays as the same micro-tube was used for the two reverse transcriptions. Lately, all the respiratory diseases of sheep and goats could be diagnosed by a single-step multiplex RT-qPCR including PPRV, *Capripoxvirus*, *Pasteurella multocida*, and *Mycoplasma capricolum* subsp. *capripneumoniae* (Settypalli et al., 2016).

Loop-mediated isothermal amplification

The LAMP applied for PPR diagnosis has a higher sensitivity than RT-PCR. The one-step LAMP technique is applicable for the diagnosis of PPR and reduces the possibility of sample contamination and will be a hopeful tool in low-financed laboratories (Venkatesan et al., 2015).

Serological diagnosis

Virus neutralization test

VNT is the prescribed test for international trade. It was applied as a confirmatory test for the differentiation between RP and PPR, and for PPRV antibodies detection in serum samples (Santhamani et al., 2016). Although PPRV is antigenically related to the RP virus, PPRV could be differentiated serologically from it (Munir, 2014).

Competitive ELISA

Competitive ELISA showed a high specificity (99.85%) and sensitivity (91.5 %) in the detection of PPRV antibodies in tested sera when compared with VNT (Abubakar et al., 2015; Mahmoud and Galbat, 2017). The market contains patent kits that have a high indicative sensitivity and specificity (92.2 % and 98.4 %, respectively) (Santhamani et al., 2016).

Hemagglutination assay

HA has a higher sensitivity than AGID in the diagnosis of PPR (Abubakar et al., 2011). This result agrees with the obtained results by Nussieba et al. (2008). Furthermore, the HA test is a rapid, simple, inexpensive, and reliable confirmatory test for the diagnosis of PPRV (OIE and FAO, 2015). Table 1 illustrates tests commonly used in the diagnosis of PPR.

Table 1. The different diagnostic tests commonly used in the diagnosis of Peste des Petits Ruminants (OIE and FAO, 2015)

Diagnostic tests/tools	Detection target	Application	Advantages	Disadvantages	Relative sensitivity/ detection limit
Virus isolation	Virus	Gold standard test	Useful for virus repository, primary step for virus characterization	Live virus, cell culture facilities required, less sensitive than RT-PCR	More sensitive than sandwich ELISA
Immunocapture ELISA/ sandwich ELISA	N protein	Diagnosis, clinical surveillance	User-friendly test, Preliminary vaccine quality control	Less sensitive than RT-PCR and RT-PCR-ELISA	^{1.9} 10 TCID ₅₀ /mL 500 TCID ₅₀ /mL
Dot-ELISA	M and N protein	Diagnosis	Easy and quick	Less sensitive than sandwich ELISA	82 % compared to sandwich ELISA

One-step multiplex RT-PCR	M and N genes	Diagnosis	Sensitive test, potential for Routine diagnosis	May not be economical for routine diagnosis	100 µg of RNA
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CONTROL OF PESTE DES PETITS RUMINANTS DISEASE

Prevention and control

The PPR is an important animal disease and its control reflects the economic status of Africa and Southern Asia. Vaccination is the main way to avoid PPR infection. African countries, including West Africa, have succeeded in the control of PPR by the use of the vaccine. As the PPR and RP are antigenically similar, so a live attenuated vaccine prepared from RP was used in the vaccination of goats against PPR and this led to one year of protection (Singh and Bandyopadhyay, 2015). Therefore, different parts of the world controlled PPR by using the tissue culture RP (TCRP) vaccine. This TCRP vaccine is used to protect small ruminants against PPR. Later on, the use of the TCRP vaccine was stopped worldwide to eradicate the RP (OIE and FAO, 2015). The current attenuated vaccines are heat-sensitive, so continuous uninterrupted maintenance of the cold chain until an application to the animal is recommended. Other currently commercially available vaccines are present in freeze-dried form. They are stable for at least two years at 2-8°C and for several years at -20 °C. After reconstitution of the vaccine, it must be utilized within 30 minutes (OIE and FAO, 2015).

The proper control of PPR depends on the solid support of the diagnostic methods and the proper vaccination program. The accessibility of live attenuated tissue culture vaccines and many diagnostic techniques/kits for the diagnosis of PPR facilitate the establishment of a good control program. For controlling the disease in a free area, a stamping out policy is applied, but in a country, like Egypt, this policy is not economic, so PPR control depends primarily on the fast and exact diagnosis or surveillance/monitoring and application of rapid vaccination program (Soltan and Abd-Eldaim, 2014). All the needed elements for a good control program are available in Egypt like an effective vaccine, availability of the diagnostic kits, sero-surveillance, and good infrastructures. Management decisions and control strategies need the development of rapid and accurate diagnostic tools. Immunoenzymatic assays are advantageous because they are cheap, reliable, and quick to perform. Thus, ELISA may be a useful tool in large-scale screening and eradication programs giving insight into the local viral infection status (Ghazy et al., 2007).

Vaccines for peste des petits ruminants

Tissue culture live attenuated vaccine

Tissue culture vaccine was the first produced vaccine for the protection against PPR. It developed from live attenuated Nigerian strain PPRV Nig. 75/1 that has been cultured for 63 passages onto Vero cells. It provokes long-standing immunity for up to 3 years (Diallo et al., 1989; Diallo et al., 2007). Under field conditions, the vaccine was safe for sheep and goats and gave immunity to 98% of the immunized animals even pregnant ones (Diallo et al., 1989). However, the major disadvantage of this type of vaccine is the thermo-liability and inability to distinguish between the vaccinated and naturally infected animals (Venkataramanan et al., 2005). Newborns of vaccinated dams should vaccinate at the age of 4-6 months. Meanwhile, newborns of non-vaccinated dams should vaccinate at the age of two months. The booster dose is recommended after one year.

Recombinant vaccines

The bases of this type of vaccine depend on the insertion of one or both of the F and H glycoproteins producing genes extracted from PPRV RNA into *Capripoxvirus*. The resulting combined vaccine induces protection against PPR and capripox (Berhe et al., 2003). The use of such a vaccine is valuable in regions indicating mixed infection with PPR and sheep or goat pox. Again, the major disadvantage of this type of vaccine is the inability to distinguish between the vaccinated and naturally infected animals (Venkataramanan et al., 2005).

Marker vaccine

In this type of vaccine, a modified live attenuated virus vaccine was developed (Singh et al., 2015). RP vaccine virus genome can be used as the main template for the vaccine besides the replacement of the M, F, and H genes of PPRV. The subsequently produced vaccine is a harmless and active vaccine to protect animals against virulent challenges with PPRV (Mahapatra et al., 2006). In addition, EHV-1 vaccine marker development would help the differentiation between the wild-type virus infection and the mutant virus immunization (Ata et al., 2018).

Vaccines for peste des petits ruminants in Egypt

The vaccination program in Egypt is annual and applied before the season of the disease occurrence, which is usually in September. The Veterinary Serum and Vaccine Research Institute, Cairo, Egypt produces the used vaccine. It

is a tissue culture attenuated PPR vaccine. It is a free obligatory vaccine for small ruminants. It is applied around the foci of infection (ring vaccination) and as an obligator in case of epidemics. The Center of Food Security and Public Health (CFSPH, 2015) discusses the important vaccines used for PPR as shown in Table 2.

Table 2. The commercially available vaccines for Peste des petits ruminants in Egypt

Producer	Product name	Type	Strain	Adjuvant	Licensed countries
Veterinary Serum and Vaccine Research Institute	Peste Des Petits Ruminants Vaccine (PPR-N75/1)	Live	Nigeria 75/1	Not Available	Egypt
M.C.I. Santé Animale	LYOPOX PPR (Sheep & Goat Pox, Peste des Petits Ruminants)	Live	Romanian (Pox) Nigeria 75 (PPR)	None	Egypt and other
	OVIVAX PPR	Live	Nigeria 75/1	None	Egypt and other
Jordan Bio-Industries Center (JOVAC)	Pestevac	Live	Nigeria 75/1	Non	Egypt and other

Source: The Center of Food Security and Public Health (2015)

Treatment

Peste des petits ruminants like all other viral diseases have no specific treatment. However, affected animals should receive antibiotics (long-acting oxytetracycline, chlortetracycline) to combat secondary bacterial infections, and antidiarrheal drugs with supportive therapy for 5-7 days. The symptomatic treatment reduces the severity of the disease and diminishes the economic losses (OIE and FAO, 2015). Moreover, dietary supplementation with natural antioxidants such as *Nigella sativa*, black cumin seeds, or multi-nutrient antioxidants provides potent immune cell function and has an antibacterial effect against secondary bacterial infections (Abou-Zeina et al., 2013).

CONCLUSION

Sheep and goats represent the essential source of meat and wool production in some countries. Sheep and goat husbandry economically depend on growing these animals in large numbers in a small area (intensive breeding). Several sheep and goat diseases that hinder the intensification of animal husbandry create complex animal health and production problems. Many conventional and modern techniques are used for the diagnosis of PPR. Numerous conventional techniques include isolation on cell culture, serological tests, and immunohistochemical assays usually used in the identification of viral pathogens. However, modern molecular diagnostic techniques are more valuable, more sensitive, fast, and high confident results. Novel methodology such as PCR, DNA probe, and nucleic acid sequencing deliver a detailed understanding of the exact diagnosis and discrimination of the present and emerging diseases. Modern diagnostic tools are urgently needed not only for diagnosis but also for monitoring and controlling viral disease programs. The control program relies mainly on hygiene, vaccination, sanitation, and effective quarantine measures.

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Authors' contribution

All authors, namely Mahmoud MA, Ghazy AA, and Shaapan contributed to developing the concept and designed the review article. Ghazy AA and Shaapan RM. Raafat M. wrote the paper and prepared it for publication.

Competing interests

The authors declare that they have no conflict of interest.

Ethical considerations

The above-mentioned was ethically cleared by authors including plagiarism checking, the article has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and there is no redundancy

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