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Peste des Petits Ruminants Virus (PPRV): Optimization of Diagnostic Procedures and Pathogenesis Studies

von Sabrina Halecker

aus Erfurt

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Aus dem Veterinärwissenschaftlichen Department der
Tierärztlichen Fakultät der Ludwig-Maximilians-Universität
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Arbeit angefertigt unter der Leitung von Univ.-Prof. Dr. Gerd Sutter

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For my family and friends.

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I INTRODUCTION

Peste des petits ruminants (PPR) is a disease of small ruminants causing enormous economic losses in the affected regions (Kumar et al., 2014). During the past few decades, the disease has spread rapidly to previously PPR-free countries in North and East Africa, East Asia as well as to European countries (De Nardi et al., 2012; Donduashvili et al., 2018; Kamel and El-Sayed, 2019; Muniraju et al., 2013; OIE, 2019b; Shatar et al., 2017; Spiegel and Havas, 2019). In 2015, the Office International des Epizooties (OIE) and the Food and Agriculture Organization of the United Nations (FAO) launched an ambitious multilevel program for the global elimination of PPR virus (PPRV) until 2030 (Cameron, 2019). In this context, several efforts were made to improve control and diagnostic measures (Jones et al., 2016). Various authors suspect that the disease is underdiagnosed in several regions due to the eligible differential diagnoses (Balamurugan et al., 2014; Luka et al., 2012; Torsson et al., 2017). Thus, regarding the optimization of diagnostic tools, it seems to be desirable to develop diagnostic methods, which are ready-to-use in the field as point-of-care (POC) tests with a good sensitivity and specificity as well as to involve the detection of further pathogens clinically similar to the signs of PPRV. Relevant pathogens that should be included in the diagnostic clarification are foot-and-mouth disease virus (FMDV), goatpox virus (GTPV) and parapoxvirus ovis as well as *Mycoplasma capricolum subsp. capripneumoniae* (Mccp), Pasteurella species or bluetongue virus (Adedeji et al., 2019; Kumar et al., 2016; Saravanan et al., 2007; Torsson et al., 2017).

The aim of the present study was first to analyse differences concerning virulence, clinical manifestation and pathogenesis of two different peste des petits ruminants virus (PPRV) isolates in goats of German breed. One isolate caused severe clinical signs in mountain gazelles near Dubai (SMRV/UAE/2018/V135/Dubai), United Arab Emirates (UAE), while the other isolate (SMRV/IND/2013/V242.5/Shahjadpur) was obtained from clinically diseased goats in Shahjadpur, India. A second focus was the validation of commercially available rapid tests for the detection of PPRV. Two antigen lateral flow devices (LFDs) as well as one antigen ELISA were investigated.

II LITERATURE REVIEW

1 The genus *Morbillivirus*

1.1 Representative morbilliviruses

Peste des petits ruminants virus (PPRV) is one out of seven morbilliviruses listed by the International Committee on Taxonomy of Viruses (ICTV; see Table 1) (ICTV, 2020c). A closely related representative of PPRV was the rinderpest virus (RPV) which showed a fatal disease in cattle and was eradicated globally in 2011 (Moutou, 2014).

Table 1 Representatives of the genus of *Morbilliviruses* according to the ICTV.

Virus name	First description	Main hosts	References
peste des petits ruminants virus	1942	goats and sheep	(Mornet et al., 1956)
rinderpest virus	more than 10,000 years ago	ungulates (eradicated in 2011)	(Moutou, 2014; Tounkara and Nwankpa, 2017)
feline morbillivirus	2012	cats	(Sutummaporn et al., 2019; Woo et al., 2012)
canine distemper virus	1746	carnivores	(Martinez-Gutierrez and Ruiz-Saenz, 2016; Quintero-Gil et al., 2019)
phocine distemper virus	1988	seals	(N.N., 1988; Osterhaus and Vedder, 1988)
porpoise morbillivirus	1988	porpoises	
dolphin morbillivirus	1990	dolphins	(Domingo et al., 1990; Duignan et al., 1995; Groch et al., 2014; Jacob et al., 2016; Kennedy et al., 1988; Sierra et al., 2016; West et al., 2013)
pilot whale morbillivirus	1995	pilot whales	
beaked whale morbillivirus	2013	beaked whales	
guiana dolphin morbillivirus	2014	guiana dolphin	
measles virus	around 900 AD	human	(Andres, 2006; Volkmer, 2015)

Besides the morbilliviruses of livestock (PPRV and RPV), there are also other important viral diseases concerning the domestic small animal population (cats, dogs) and carnivores, respectively (Table 1). A recently discovered morbillivirus infecting cats was first described in 2012 and named "feline morbillivirus" (Sutummaporn et al., 2019; Woo et al., 2012). The canine distemper virus (CDV) affects a broad-range of hosts including domestic dogs as well as foxes, raccoons, martens, raccoon dogs, badgers, wolves and many other wildlife species (Martinez-Gutierrez and Ruiz-Saenz, 2016). In the case of marine mammal morbilliviruses, the phocine distemper virus (PDV), which was responsible for recurring, massive outbreaks in seals in 1988 and 2002 (Duignan et al., 2014; Muller et al., 2008; N.N., 1988), is distinguished from the cetacean morbilliviruses consisting of five strains (Jo et al., 2018; Sierra et al., 2016). The cetacean morbilliviruses affect mainly marine mammals as porpoises and several species of dolphins and whales (Table 1). According to the latest data, there are first indications that new representatives of the genus *Morbillivirus* have been found in bats and rodents (Drexler et al., 2012). Phylogenetic relationships between members of the genus *Morbillivirus* are shown in Figure 1.

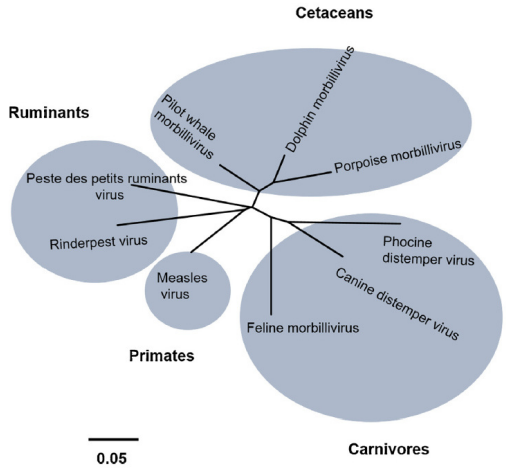


Figure 1 Phylogenetic relationships between members of the genus *Morbillivirus* (Parida et al., 2016). [For permission rights see chapter Supplement, page 63].

1.2 Taxonomy and classification of peste des petits ruminants virus (PPRV)

According to the most current taxonomy of the ICTV (ICTV, 2020c), PPRV is categorized into the order *Mononegavirales*, of the family *Paramyxoviridae* and the genus *Morbillivirus*. In 2017, the virus species *PPRV* was renamed to the current taxonomic name *Small ruminant morbillivirus* (Amarasinghe et al., 2017). Worldwide, one serotype is known for PPRV, which is divided into four genetic lineages (I, II, III, IV). Phylogenetic analyzes and epidemiological data show that lineage IV is at the moment of great impact (Figure 2). Differentiation of the lineages is characterized by partial genome sequence analysis of the nucleocapsid (N) and the fusion (F) gene, respectively (Parida et al., 2016).

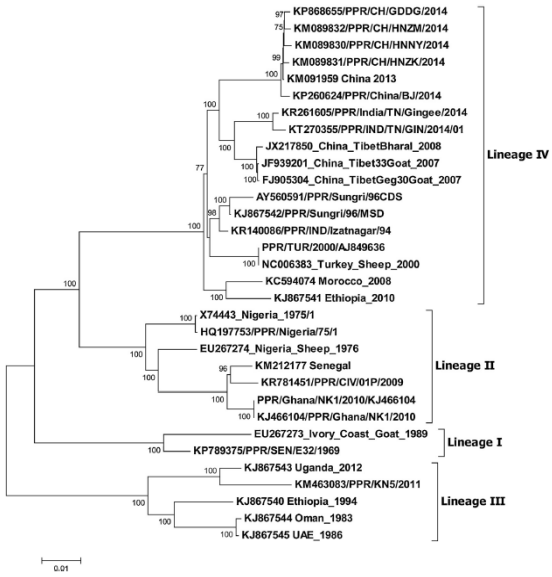


Figure 2 Phylogenetic tree of PPRV isolates based on full-genome sequences showing the relationship of PPRV isolates selected and the relationship of the four lineages among each other (Parida et al., 2016). [For permission rights see chapter Supplement, page 63].

1.3 Virion structure and genome organisation of PPRV

Virion particles of PPRV are enveloped with a pleomorphic shape and a size of 150 to 700 nm (Bourdin and Laurent-Vautier, 1967; Gibbs et al., 1979). The linear single-stranded, negative-sense RNA genome of PPRV consists of 15,948 nucleotides (Bailey et al., 2005). Six genes encode for eight proteins (Figure 3) driven by RNA editing and an alternative open reading frame. In detail, six structural (nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), large (L)) and two non-structural proteins (C and V) are distinguished (Kumar et al., 2014; Libeau, Diallo, and Parida, 2014).

The genomic RNA of PPRV is encapsulated by the N protein which is the most abundant viral protein transcribed among all genes of PPRV (Bailey et al., 2007; Kumar et al., 2014; Yunus and Shaila, 2012). The interaction of N, P and L proteins leads to the formation of the ribonucleoprotein complex spanning the entire genome of PPRV and protecting it from endonuclease digestion (Parida et al., 2015; Sourimant and Plemper, 2016). Besides, P and L proteins are main components of the RNA-dependent RNA polymerase, thus responsible for viral replication and transcription (Bailey et al., 2007; Kumar et al., 2014). The P protein has a high variability in its nucleotide sequence compared to the other proteins (Sourimant and Plemper, 2016). According to the mRNA gradient, the amount of L protein is the lowest for all proteins (Yunus and Shaila, 2012). The M protein is located under the virus envelope and attaches the two surface glycoproteins F and H proteins in place. Furthermore, the M protein interacts also with the ribonucleoprotein complex and facilitates a connection between all these proteins enabling the formation and budding of new virus particles (Haffar et al., 1999). F and H proteins are embedded in the viral envelope and are responsible for the viral uptake into the host cells via adhesion and fusion processes (Rahaman et al., 2003). C and V proteins have regulatory functions with regard to the modulation of the RNA polymerase and RNA synthesis, virus replication and virulence factors. The sequences of both proteins are located on the P protein (Kumar et al., 2014).

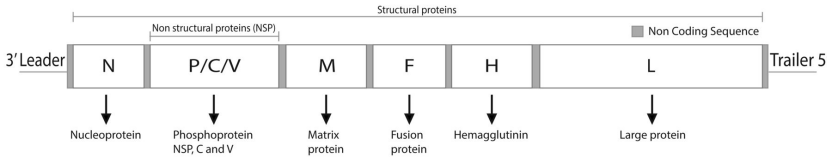


Figure 3 Schematic organisation of the PPRV genome containing six genes (Libeau et al., 2014). [For permission rights see chapter Supplement, page 63].

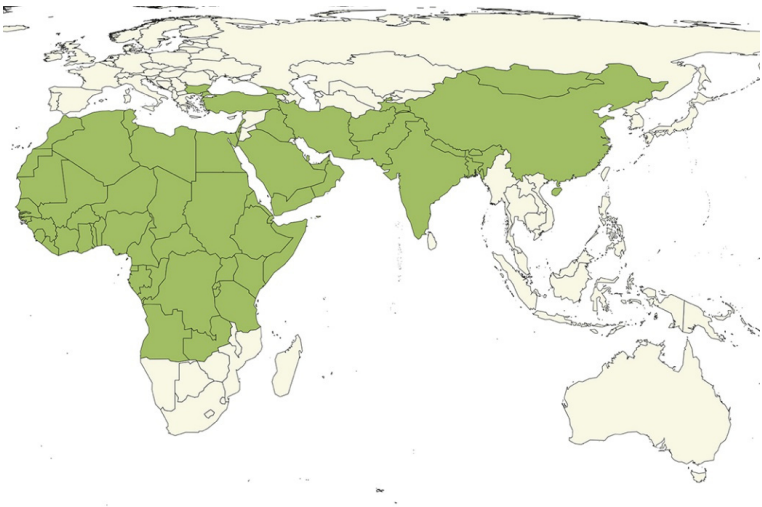
The efficient replication of the viral genome follows the “rule of six”, which means that the RNA-dependent RNA polymerase only works correctly if the total number of nucleotides represents a multiple of six nucleotides (6 n + 0) (Bailey et al., 2005; Sourimant and Plemper, 2016; Vulliemoz and Roux, 2001). Thus, genome variations are limited to insertion or deletion of hexamers (Kolakofsky et al., 2005; Rima, Collin, and Earle, 2005).

2 Impact of PPRV on livestock production and wildlife

2.1 Global distribution

PPRV was first identified in 1942 at the Ivory Coast. Since then, it has spread widely across Africa, the Middle East and Asia (Figure 4) (Banyard et al., 2010). Currently, PPRV occurs in over 70 countries affecting 80 % of the sheep and goat population in the world (Baron et al., 2017).

Figure 4 Global distribution of PPRV as of April 2019 (Cameron, 2019). [For permission rights see chapter Supplement, page 63].



From a global perspective, PPRV has expanded considerably in recent decades. Various new PPRV outbreaks in previously PPRV-free countries occurred in Kenya in 2006, in Uganda in 2007 and in Tanzania in 2008 (Dundon et al., 2017; Luka et al., 2012; Spiegel and Havas, 2019; Torsson et al., 2016). Nearly at the same time, in 2008, the first outbreak of PPRV in Morocco affecting mainly alpine goats was recognized (Hammouchi et al., 2012; Muniraju et al., 2013). Seven years later, the goat and sheep population in Morocco was overrun by another wave of PPRV infections with an isolate that differs significantly from that of 2008 (Fakri et al., 2016). In 2010, the disease was notified for the first time in the neighbouring country Algeria caused by an isolate closely related to the Moroccan isolate from 2008 (De Nardi et al., 2012). Several years

later, PPR-cases occurred in the naïve sheep and goat population of Georgia and Mongolia, respectively (Donduashvili et al., 2018; OIE, 2019c; Rajko-Nenow et al., 2017; Shatar et al., 2017). In summer 2018, PPRV infections in small ruminants were detected for the first time in the European Union in Bulgaria (Kamel and El-Sayed, 2019; OIE, 2019b).

2.2 Host species and epidemiology

The main hosts suffering from PPR are domestic small ruminants. Originally, the disease was assumed to be triggered by RPV that also could affect goats and sheep (Parida et al., 2015). Remarkably, PPRV infections in sheep have been less severe than in goats (EFSA, 2015; Wernike et al., 2014). The latest findings show that the sole consideration of the main hosts regarding the spread of PPRV is not sufficient (Aziz Ul et al., 2018; Munir, 2014; Rahman et al., 2020).

Large ruminants including cattle and water buffalos have been discussed as potential hosts for PPRV (Abubakar et al., 2017). Reports about natural infections and clinical disease in cattle and buffalos are rare, while multiple evidence of seroconversion in cattle exist (Rahman et al., 2020). Experimentally infected cattle showed no or only less clinical signs, no shedding of infectious PPRV and no transmission to contact animals (Couacy-Hymann et al., 2019; Schulz et al., 2019). Thus, cattle have been considered as dead-end hosts for PPRV because they are susceptible to PPRV without transmitting the virus to further animals (Rahman et al., 2020). The reports concerning the clinical manifestation and epidemiological impact of camelids are contrary. Clinical diseases occurring in camelids were described to be different in their severity ranging from subclinical, acute to peracute forms of infection (Khalafalla et al., 2010; Zakian et al., 2016). However, a variety of seroprevalence studies indicated a low serological prevalence in camelids (Fakri et al., 2019), even in close proximity to small ruminants (Hemida and Al-Ghadeer, 2019). In contrast, experimentally inoculated camelids showed no clinical signs and no virus shedding in excretions and secretions (Fakri et al., 2019; Schulz et al., 2019). There was also no transmission to contact animals. The latter findings indicate a strong evidence that camelids are also dead-end hosts (Schulz et al., 2019). A potential role in transmission and maintenance of PPRV have been discussed for suids. Animal experiments showed that domestic pigs and wild boars could be infected with PPRV, became mild to moderately ill and were able to transmit the virus to contact pigs and goats (Schulz et al., 2018).

Besides, a wide range of wild ungulates such as gazelles, wild goats and sheep, bharals, ibex, antelopes and deer have been described to be susceptible for PPRV (Bao et al., 2011; Hamdy and Dardiri, 1976; Hoffmann et al., 2012; Kinne et al., 2010; Marashi et al., 2017). The epidemiological impact of wildlife species is not clearly understood (Fernandez Aguilar et al., 2020; Fine et al., 2020; Munir, 2014). However, several reports indicate that PPRV outbreaks in wildlife populations were driven by domestic animals (Abubakar et al., 2011b; Bao et al., 2011; Mahapatra et al., 2015). Up to now, there is little evidence of transmission in the opposite direction ("spillback"), although theses epidemiological scenarios are also discussed (Aziz Ul et al., 2018; Fenton and Pedersen, 2005; Kinimi et al., 2020; Munir, 2014; Nugent, 2011). Overall, PPRV has to be considered as a serious threat to endangered wildlife species (Abubakar et al., 2011b; Marashi et al., 2017; Pruvot et al., 2020).

For the African conditions, various political, social and natural events are held responsible for the spread of PPRV, since they led to animal movements and trade. In detail, cattle trade, cultural events, animal husbandry, nomadism, feuds between pastoralist groups, dowry for the wedding, civil wars and dubious elections as well as droughts leading to refugee movements are pointed out as major factors that benefit the spread of PPRV (Spiegel 2018).

2.3 The idea of global PPRV eradication and control measures

Due to the socioeconomic impact of PPRV infection in the affected countries as well as the successful global eradication of rinderpest (RP) in 2011, efforts are also being made to eradicate PPRV (Diallo, 2006; Kumar et al., 2014). For this purpose, OIE and FAO passed an extensive program to achieve this goal by 2030 (Cameron, 2019; Jones et al., 2016). At an early stage of eradication, monitoring the current health situation of the animal population and targeted control measures such as vaccination should be used to curb the spread of PPRV and to achieve PPRV-free populations (Jones et al., 2016). In endemic areas, vaccination of the animals is sought (Banyard et al., 2010; Liu et al., 2014). For this purpose, homologous vaccines are currently available such as "Nigeria 75/1", which is the most used vaccine strain in Africa. Besides, further live-attenuated PPRV-vaccines such as Sungri 96, Arasur 87, Coimbatore 97 or Egypt/87 are available (Bora et al., 2018b; Liu et al., 2014).

The above mentioned live-attenuated vaccines have the problem to be less thermostable and are degraded when transported without cold chain (Bora et al., 2018b). Therefore, several attempts were made to develop thermostable vaccines for their use under tropical and subtropical climate conditions. Thus, chemical stabilizers (lactalbumin hydrolysate-sucrose, Weybridge medium, lactalbumin hydrolysate-manitol, buffered gelatin-sorbitol, trehalose dehydrate, stabilizer E) were e.g. used to prepare lyophilized vaccines. Another approach is to passage virus isolates at relatively high temperatures in order to reduce their temperature sensitivity. The vaccine "Jhansi 2003" was developed following this approach and proved to be safe, immunogenic, efficacious and more thermostable (Liu et al., 2014).

The new generation of PPRV vaccines also included the strategy of differentiation of infected from vaccinated animals (DIVA). DIVA vaccines can be developed as positive (contain an additional heterologous epitope or domain) and negative markers (absence of a homogenous epitope or domain). Several PPRV DIVA vaccines were developed based on viral vector vaccines, chimeric virus vaccines, vaccines using reverse genetics, subunit vaccines and nucleic acid vaccines, but they have not been commercialized up to now (Liu et al., 2014). Besides, combined vaccines containing both PPRV and sheeppox virus was developed enabling the control of two infectious diseases simultaneously (Berhe et al., 2003; Chaudhary et al., 2009).

At a later stage of eradication, when the further spread of the disease and the circulating of the virus is stopped, effective surveillance programs are needed to proof the absence of the disease or virus in all susceptible animals for reaching the OIE official status "free from infection" (Couacy-Hymann et al., 2005).

3 Clinical significance of PPRV infection

3.1 Clinical manifestation and pathogenesis

PPRV is a disease that must be notified to the OIE (OIE, 2020). Morbidity and mortality rates are high ranging from 90-100 % in naïve populations of small ruminants, decreasing to 20 % in endemic regions (Abubakar et al., 2017). First clinical signs appear normally 4 to 6 days after an infection with PPRV, but the incubation period is variable and can last 3 to 14 days (Parida et al., 2015). Characteristic clinical signs shown in infected goats and sheep are manifested in the respiratory and the gastrointestinal tract and, in the case of pregnant animals also in the reproductive tract. Erosive lesions of the ocular, nasal and mouth mucous membranes as well as oculo-nasal discharges combined with labored breathing are described. The disease is associated with high fever up to 41 °C, loss of appetite and weight loss of the animals due to watery diarrhea (Parida et al., 2015). Due to its lymphotropic character, PPRV causes a severe immunosuppression in infected animals leading to a decrease in the antibody response and facilitating secondary infections (Rajak et al., 2005).

3.2 Relevant pathogens in differential diagnostics

FMDV, GTPV, parapoxvirus ovis, Mccp, Pasteurella species and bluetongue virus have to be considered as differential diagnosis to PPR due to the very similar clinical signs in small ruminants (Santhamani, Singh, and Njeumi, 2016).

Foot-and-mouth disease is a highly contagious disease of cattle, pigs, sheep, goats, buffaloes and numerous wildlife species (Brito et al., 2017; Jamal and Belsham, 2013). The first description dates back to 1514 (Jamal and Belsham, 2013). FMDV belongs to the family *Picornaviridae* and the genus *Aphthovirus* (ICTV, 2020a). Serologically, a distinction between seven serotypes (Asia-1, A, O, C, SAT 1, 2, 3) is made. Clinically, affected animals show pyrexia, anorexia, salivation, vesicles around nose and mouth as well as in the interdigital spaces (Jamal and Belsham, 2013). Due to the pain induced by the lesions, many infected animals show lameness. The severity of the clinical signs can vary widely depending, among other things, on the animal species and especially small ruminants like sheep often show very mild clinical signs (Alexandersen et al., 2003).

Parapoxvirus ovis infection, also known as Orf or contagious ecthyma, is a zoonotic and self-limiting disease of the skin and mucous membrane which occurs mainly around the areas of nose, lips and mouth (Hosamani et al., 2009). It is a benign disease that spontaneously disappears in humans and animals within 6-8 weeks (Bergqvist, Kurban, and Abbas, 2017). The lesions occur initially as erythema developing vesicles covered with scab. Nevertheless, it is a painful disease that affects especially lambs and kids while suckling and grazing (Spyrou and Valiakos, 2015). The causative agent belongs to the family *Poxviridae* and to the genus *Parapoxviruses* (ICTV, 2020d).

GTPV is one out of the three virus species categorized in the genus *Capripoxvirus* within the family *Poxviridae* (ICTV, 2020b). The disease shows characteristic pox lesions in the skin on the entire body surface associated with fever, fatigue, reduced feed intake and often lesions of the lung (Bora et al., 2018a; Ramakrishnan, Santhamani, and Pandey, 2017; Tulman et al., 2002). Decreased productivity as a reduced milk yield, weight losses, abortion and impairments to wool and hides are associated with that disease (Babiuk et al., 2008). In naïve populations, morbidity and mortality rates vary from 75-100 % and 10-58 %, respectively (Rao and Bandyopadhyay, 2000).

Contagious caprine pleuropneumonia (CCPP) is listed by the OIE and caused by the gram-negative bacterium Mccp which belongs to the class *Mollicutes*. The disease is spread mainly in African and Asian countries (Nicholas and Churchward, 2012). The main hosts are goats but sheep, ibexes, mouflons, gazelles, antelopes, gerenuks and further deer species can also be infected. Morbidity and mortality rates can be very high of up to 100 % (Iqbal Yatoo et al., 2019; Nicholas and Churchward, 2012). Clinical signs of affected animals are pyrexia, nasal discharge, cough, and dyspnea. The infected animals are often lethargic and refuse to eat (Arif et al., 2007).

4 Diagnostic methods for the detection of PPRV

The diagnosis of PPR-suspected animals has to be confirmed by laboratory diagnostics due to the similarity of the clinical signs with other relevant diseases (as described in chapter 3.2), the overlapping of clinical signs in mixed infections and various clinical manifestations in PPRV-infected animals (Santhamani et al., 2016). For laboratory confirmation of clinical cases, a broad range of diagnostic tools is available (Kinimi et al., 2020; OIE, 2019a). Figure 5 summarizes several commonly used diagnostic methods for the detection and characterization of PPRV infections.

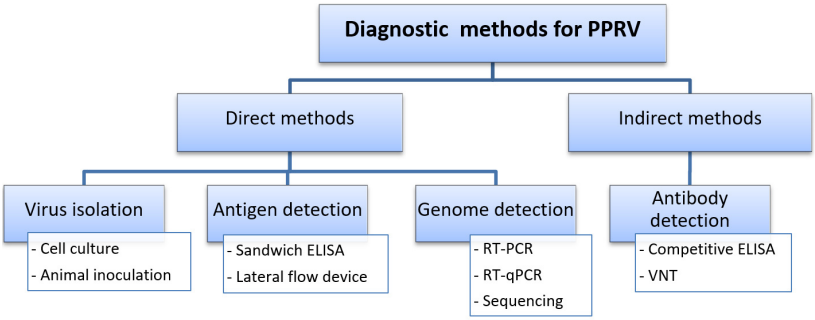


Figure 5 Classification of diagnostic methods for PPRV. The here presented methods were also used in the thesis. ELISA = enzyme-linked immunosorbent assay, RT-PCR = reverse transcription polymerase chain reaction, RT-qPCR = real-time quantitative PCR, VNT= virus neutralization test.

4.1 Direct methods of PPRV detection

Cell Culture

Virus isolation of PPRV is the gold standard test for confirmatory diagnostics (Balamurugan et al., 2014; Santhamani et al., 2016). Therefore, established cell lines as the African green monkey kidney cells (Vero) and also Vero cells expressing the canine receptor signaling lymphocyte activation molecule (SLAM), so called Vero dog-SLAM (VDS) cell line, are commonly used

(Lefèvre and Diallo, 1990; von Messling et al., 2003). Characteristic cytopathic effects (CPEs) as indicator for a successful infection of the cells are rounding of the cells, berry-shaped clustering followed by fusion of the cells and formation of syncytia (Figure 6). Gradually, the cell layer becomes detached (Lefèvre and Diallo, 1990; Santhamani et al., 2016). When using Vero cells, several blind passages might be necessary until a CPE can be observed (OIE, 2019a). In order to increase the success rate of virus isolation, cell lines expressing the morbillivirus receptor SLAM (CD150) were established (von Messling et al., 2003). Regarding virus-host interactions, the SLAM receptor plays a crucial role for the uptake of PPRV to immune cells like lymphocytes, macrophages and dendritic cells that express the SLAM receptor (Kumar et al., 2014). Besides, the application of cell culture needs time, appropriate laboratory equipment and laboratory space in a high containment facility, and is elaborate for routine diagnostics (Balamurugan et al., 2014; Santhamani et al., 2016). Therefore, other techniques were established for routine diagnostics (Saliki et al., 1994; Santhamani et al., 2016).

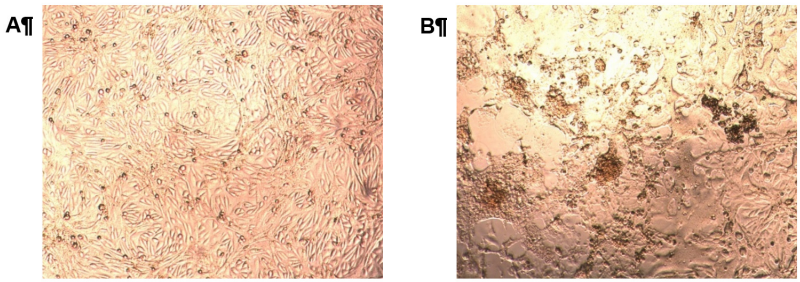


Figure 6 Appearance of native VDS cells (A) and VDS cells showing PPRV-induced CPE two days after an inoculation (B).

Antigen enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assays (ELISAs) are tests based on antigen-antibody reactions (Aydin, 2015). These tests are user-friendly, can have a high throughput of samples, and are suitable for automation (O'Kennedy et al., 1990; Santhamani et al., 2016). ELISAs can be used for both antigen and antibody detection (see also chapter 4.2) (Aydin, 2015). The specificity of antigen ELISAs is often high, while the sensitivity could be reduced, depending on the amount

of antigen in a sample (Diop, Sarr, and Libeau, 2005; Singh et al., 2004a; Zhang et al., 2019). For antigen detection of PPRV several ELISA test formats are available (Santhamani et al., 2016).

One commonly used ELISA format for the detection of viral antigens is the so-called sandwich ELISA (S-ELISA). The underlying principle of an S-ELISA is rested on capturing the antigen to be determined between two layers of antibodies (Aydin, 2015). Therefore, one layer of PPR-specific antibodies (capture antibody) is coated on a solid phase to which the clinical sample containing the target antigen is added (Libeau et al., 1994). Afterwards, detection antibodies bind to the free antigen binding sites varying from that of the capture antibody (Aydin, 2015; O'Kennedy et al., 1990). A colour signal is elicited by the interaction of a chromogenic substrate that is added to the assay and an enzyme attached to the detection antibody (Crowther, 2009). Enzymes that are used for labelling antibodies are alkaline phosphatase, horseradish peroxidase, acetylcholinesterase, ß-galactosidase, glucose oxidase or urease (Aydin, 2015; O'Kennedy et al., 1990). Various variations of these S-ELISAs are known (Santhamani et al., 2016). A brief overview of several antigen ELISAs for the detection of PPRV is given below.

An immunocapture ELISA (IC-ELISA) developed by Libeau et al, 1994, is based on the use of monoclonal antibodies (mAbs) directed against the N protein of PPRV. This commercially available assay is designed as direct sandwich ELISA (S-ELISA) with a diagnostic sensitivity of around $10^{1.9}$ $TCID_{50}$/ml (Crowther, 2009; ID.Vet, 2019b; Libeau et al., 1994). The IC-ELISA is a more sensitive detection method compared to the agar gel immunodiffusion (AGID) test or the haemagglutination (HA) test (Abraham and Berhan, 2001; Abubakar et al., 2011a). The diagnostic sensitivity and specificity for the IC-ELISA is specified as 84.6 % and 96.7 %, respectively (Diop et al., 2005).

Another ELISA for the detection of PPRV antigen and commonly used in India for screening and diagnosis of PPR (Balamurugan et al., 2012a; Santhamani et al., 2016) is the indirect sandwich ELISA (Crowther, 2009) by Singh and co-workers (Singh et al., 2004a). As capture antibodies, polyclonal rinderpest hyperimmune sera are used to coat the ELISA plates. For the detection of the antigen, a primary monoclonal antibody specific for PPRV and raised against the N protein (Singh et al., 2004a) as well as a secondary antibody (rabbit anti-mouse antibody) conjugated with peroxidase are added to the assay. Compared to the ELISA designed by Libeau et al., 1994, the diagnostic sensitivity is 88.9 % and the diagnostic specificity is 92.8 % (Singh et al., 2004a).

Antigen lateral flow devices (LFDs)

Lateral flow devices (LFDs) are rapid diagnostic devices which are variable in their size, shape and configuration (Yetisen, Akram, and Lowe, 2013). The LFD-based assays used in our studies rely on antigen-antibody reactions. One of these assays is available as cassette format containing the paper strip inside, and another one is designed as dipstick (Baron et al., 2014; ID.Vet, 2019a). These immune-chromatographic test systems are suited for a quick and simple use in the field obtaining results within 20 minutes and with a minimum of equipment necessary for their application (OIE, 2019a; Posthuma-Trumpie, Korf, and van Amerongen, 2009; Rozand, 2014). In fact, the interpretation of these test systems can be done by eye without any further instruments (Rozand, 2014). A high throughput of varying sample types is feasible using those LFDs (Posthuma-Trumpie et al., 2009; Rozand, 2014). Nevertheless, the LFDs exhibit a relative low diagnostic sensitivity while the diagnostic specificity is high (Zhang et al., 2019). Immuno-chromatographic LFDs are divided into sandwich and competitive formats (Ferris et al., 2009; Laitinen and Vuento, 1996). LFDs normally use stripes out of nitrocellulose membranes as solid phase that consists of a sample pad, a conjugate pad, a detection zone containing a test line and a control line, and an absorbent pad at the end of the stripe (Figure 7). The sample prepared with buffer solution is applied on the sample pad and is then carried by capillary flow to the conjugated pad (Yetisen et al., 2013). On the conjugated pad, recognition elements are dried on the paper labeled mostly with colloidal gold or latex (Posthuma-Trumpie et al., 2009). When a sample with the target antigen reaches this pad, the target antigens build a complex with the labeled antibodies and the coloured recognition elements are released in a controlled manner. The liquid migrates further to the test line where the immune complexes irreversibly interact with the immobilised antibodies and present a chromatographic signal (positive result) (Zhang et al., 2019). On the control line, the unbound conjugated particles are also bound to immobilised mAbs resulting in a coloured line which indicates a valid test (Figure 7) (Ferris et al., 2009). When using an LFD based on a sandwich format (direct assay), a coloured line is visible on both the test and the control line in the case of a positive result. In the case of a negative result, the test will provide no coloured test line, but a coloured line appears on the control field (Yetisen et al., 2013).

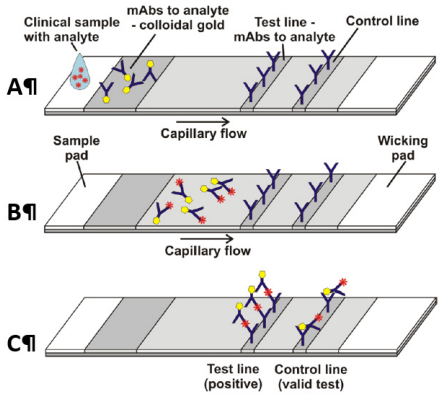


Figure 7 Operating principle of a lateral flow device (LFD) with colloidal gold as label in a sandwich LFD-format. A: Application of a clinical sample on the sample pad and flow of the sample by capillary force to the conjugated pad. B: Target antigens captured by the labelled antibodies and released to the detection zone. C: For a positive result, a coloured line is visible on both test and control line (Lee et al., 2013). [The original figure was transformed by using capital letters for the individual figures. For permission rights see chapter Supplement, page 63].

Polymerase chain reaction (PCR)

Several molecular techniques (e.g. RT-PCR, RT-qPCR, RT-PCR-ELISA, cDNA probes, reverse transcription loop-mediated isothermal amplification (RT-LAMP), reverse transcription recombinase polymerase amplification (RT-RPA), sequencing, microarrays) are available for the detection of PPRV-specific nucleic acids (Rajko-Nenow et al., 2017; Santhamani et al., 2016). In addition to the gel-based reverse transcription polymerase chain reaction (RT-PCR) which provides qualitative assertion regarding the genomic loads in a sample (Mackay, Arden, and Nitsche, 2002; Rodriguez-Sanchez et al., 2008), the real-time quantitative RT-PCR (RT-qPCR) has been implemented in diagnostics (Bustin, 2002). The latter method is able to make a quantitative statement and thus differentiate between samples with a low or a high genome load, respectively (Balamurugan et al., 2014; Wall and Edwards, 2002). For the detection of

PPRV genomes, numerous assays are established for molecular diagnostics (Balamurugan et al., 2014; Bao et al., 2008; Batten et al., 2011; Kwiatek et al., 2010; Polci et al., 2015).

4.2 Indirect methods of PPRV detection

Indirect diagnostic tools are based on the detection of specific antibodies. An advantage of this diagnostic principle is that antibodies can still be detected years after an infection or vaccination. Thus, these methods are suitable means e.g. to monitor vaccination campaigns or to carry out sero-surveillance (Santhamani et al., 2016). But there are restrictions when it comes to differentiating the origin of the antibodies, mainly if the antibodies are infection-induced or vaccine-driven (Mariner et al., 2016; OIE, 2019a).

However, in the case of a PPRV infection, H and F proteins are the major components for a protective immune response, with most of the neutralizing antibodies directed against the H protein (Diallo et al., 2007; Yan et al., 2019). Even though the N protein is produced in large amounts accompanying an infection with PPRV, the immune response against this protein does not offer sufficient protection for the animals (Diallo et al., 2007; Mitra-Kaushik, Nayak, and Shaila, 2001). Because of this facts, both N and H proteins are supposed to be the most valuable target proteins for the development of indirect diagnostic tools (Munir et al., 2012).

Antibody enzyme-linked immunosorbent assays (ELISA)

ELIASs are the most suitable assays for antibody detection, especially for larger sample numbers (Santhamani et al., 2016).

For the detection of PPRV-specific antibodies the so-called competitive ELISAs (C-ELISA) is most often applied, occasionally also designated as blocking ELISA (B-ELSA) (Alber et al., 2015). Some authors differentiate strictly between C- and B-ELISA according to their application protocols as two separate ELISA formats (Saliki et al., 1993). However, the synonymous use of both terms is usual, and therefore this nomenclature is also used in this thesis.

For the C-ELISA, PPRV-specific antigens are immobilized to a solid phase to determine the analyte (serum antibody of the sample) (Aydin, 2015). Serum sample and detection antibodies

are added to the assay; thus the antibodies can dock to the antigen binding sites (Aydin, 2015; Singh et al., 2004b). The C-ELISA is based on the principle of competition between analyte and detection antibodies for free antigen binding sites (O'Kennedy et al., 1990). In the case that the sample contains a high amount of pathogen-specific antibodies, the detection antibody is displaced from the epitope sites of the antigen. Because the ELISA is based on a chromogenic substrate which interacts only with the detection antibody, a high amount of serum antibodies is determined by a reduced colour signal (inversely proportional) (Aydin, 2015; O'Kennedy et al., 1990). Table 2 gives an overview of several antibody ELISAs designed for the detection of PPRV-specific antibodies.

Table 2 Overview of selected ELISAs for the detection of PPRV-specific antibodies (the indicated sensitivities and specificities are the result of a comparison with the gold-standard assay, the virus neutralization test).

Name of the ELISA (designed by)	designated by authors as	Target protein	Sensitivity (Se) Specificity (Sp)
(Saliki et al., 1993)	B-ELISA	H protein	Se = 90.4 % Sp = 98.9 %
(Anderson, McKay, and Butcher, 1990)	C-ELISA	H protein	not specified
(Libeau et al., 1995)	C-ELISA	N protein	Se = 94.5 % Sp = 99.4 %
(Singh et al., 2004b)	C-ELISA	H protein	Se = 92.4 % Sp = 98.4 %
(Balamurugan et al., 2007)	Indirect ELISA	not specified	Se = 80.0 % Sp = 100.0 %

Virus neutralization test (VNT)

The virus neutralization test (VNT) is described as the gold-standard for the detection of neutralizing antibodies (Couacy-Hymann et al., 2009; Santhamani et al., 2016) because it is both a highly sensitive and specific test system (Gauger and Vincent, 2014; OIE, 2019a). However, it is time-consuming and requires high demands on laboratory equipment within a high-containment facility. In brief, a twofold dilution series of the serum is incubated with precisely defined virus dilutions before it is inoculated with the cell suspension (Santhamani et al., 2016). Antibody titer determination is normally based on 50 % absence of CPE (neutralization titre ND_{50}) in the highest dilution (Alber et al., 2015).

In summary, direct methods are focusing on the detection of the pathogen itself, comprising the isolation of virus particles or the detection of viral antigens or viral nucleic acids. Besides, the indirect methods are used for the detection of virus specific antibodies in response to a PPRV infection. The methods described here are varying in their time exposure, detection window, requirements concerning the laboratory facilities as well as to the user abilities and training. Differences according to diagnostic sensitivities and specificities were also described. Some of the detection methods are applicable in various formats or are sometimes also combined. However, numerous detection methods of PPRV are well established, some are more suitable for the use under laboratory conditions and some are also applicable for the POC use. Regarding the wide range of diagnostic methods available for the detection of PPRV infections, our studies focused on POC methods. Therefore, animal inoculations were performed with two different PPRV isolates aiming to examine the pathogenesis in goats and to collect various sample materials (EDTA blood, serum, ocular, nasal, mouth and fecal swabs, fecal samples).

III OBJECTIVES

In order to enable the surveillance of clinically diseased animals at the point of care, available diagnostic tools should provide fast results with high reliability, and should concurrently be fit-for-purpose for the use in the field. In this context, the current thesis is embedded with emphasis on *in vivo* studies with two PPRV isolates for *in vivo* characterization, for the collection of reference samples, and for the comparative evaluation of several PPRV rapid detection methods for.

Objective 1: *In vivo* studies with two newly emerged PPRV strains from Shahjadpur, India and from Dubai, United Arab Emirates (UAE) for characterization and sample collection

Goats of German breeds were intranasally infected with two various PPRV isolates originating from different outbreaks, locations and hosts. We aimed to do an *in vivo* characterization of the viral isolates and to investigate influences of strain virulence, host species, and virus processing in vitro on clinical manifestations. In addition, several sample materials (EDTA blood, serum, ocular, nasal, mouth and fecal swabs) were taken to allow comparative validations and with respect to virus shedding in different excretions.

Objective 2: Validation of several direct detection methods for PPRV concerning a rapid diagnosis and a high throughput of a large amount of samples

Many efforts have been made to develop simple, quick and reliable POC diagnostics for the detection of PPRV. In this context, we strived to compare different antigen detection methods with regard to their diagnostic sensitivities and specificities as well as concerning their suitability for field use. For this, we compared a commercially available ELISA test kit (ID Screen® PPR Antigen Capture) and two LFDs (ID Rapid® PPR Antigen and PESTE-Test) using ocular and nasal swabs as well as fecal samples. As reference method, a PPRV-specific RT-qPCR (Polci et al., 2015) was used.

IV RESULTS

Comparative evaluation of different antigen detection methods for the detection of peste des petits ruminants virus

Sabrina Halecker[1], Sunitha Joseph[2], Rubeena Mohammed[2], Ulrich Wernery[2], Thomas C. Mettenleiter[3], Martin Beer[1], Bernd Hoffmann[1*]

[1]Institue of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany

[3]Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany

[2]Central Veterinary Research Laboratory, Dubai, United Arab Emirates

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Abstract

Peste des petits ruminants (PPR) is a fatal disease of small ruminants which has spread rapidly to previously PPR-free countries in recent decades, causing enormous economic losses in the affected regions. Here, two newly emerged PPR virus (PPRV) isolates from India and from the Middle East were tested in an animal trial to analyze their pathogenesis, and to evaluate serological and molecular detection methods. Animals infected with the two different PPRV isolates showed marked differences in clinical manifestation and scoring. The PPRV isolate from India was less virulent than the virus from the Middle East. Commercially available rapid detection methods for PPRV antigen (two Lateral Flow Devices (LFD) and one antigen ELISA) were evaluated in comparison with a nucleic acid detection method. For this purpose, ocular and nasal swabs were used. Due to the easy non-invasive sampling, fecal samples were also analyzed. For all rapid antigen detection methods a high specificity of 100% was observed independent of the sample matrix and dilution buffers used. Both, antigen ELISA and LFD tests showed highest sensitivities for nasal swabs. Here, the detection rate of the antigen ELISA, the LFD-PESTETEST and the LFD-ID Rapid-Test was 78%, 75% and 78%, respectively. Ocular swabs were less suitable for antigen detection of PPRV. These results reflect the increased viral load in nasal swabs of PPRV infected goats compared to ocular swabs. The fecal samples were the least suitable for antigen detection. In conclusion, nasal swab samples are the first choice for the antigen and genome detection of PPRV. Nevertheless, based on the excellent diagnostic specificity of the rapid tests, positive results generated with other sample matrices are solid. In contrast, negative test results can be caused on the reduced analytical sensitivity of the rapid antigen tests and must be treated with caution.

Keywords: diagnostic; goats; pathogenesis; virus; peste des petits ruminants (PPR); rapid detection methods; *Small ruminant morbillivirus*

Introduction

Peste des petits ruminants (PPR) is a highly contagious disease, especially in goats and sheep (Balamurugan, Hemadri, Gajendragad, Singh, & Rahman, 2014). The causative peste des petits ruminants virus (PPRV; taxonomic name: *Small ruminant morbillivirus*) is classified into the genus *Morbillivirus* in the family *Paramyxoviridae* (Amarasinghe et al., 2017). Only one serotype is known with four genetic lineages (LI-IV) (M. D. Baron, Diallo, Lancelot, & Libeau, 2016; Libeau, Diallo, & Parida, 2014). The viral genome is a linear, single-stranded, negative sense RNA of 15,948 nucleotides containing six genes. Due to different open reading frames and RNA editing eight proteins can be translated. There are six structural proteins, the nucleocapsid protein (N), the phosphoprotein (P), the fusion protein (F), the matrix protein (M), the hemagglutininneuraminidase protein (HN), the large protein (L) as well as two non-structural proteins, C and V (Kumar et al., 2014).

In naïve populations the disease induces a morbidity of up to 100 % (M. D. Baron, Diop, Njeumi, Willett, & Bailey, 2017) and a mortality of up to 80-100 %. Lower mortality rates of 20 % can be observed in endemically infected areas (Banyard et al., 2010; Pope et al., 2013). The incubation period lasts for four to six days. Infected animals show a loss of appetite associated with high fever and oculo-nasal discharge, oral lesions, bronchopneumonia, cough, dyspnea, gastroenteritis, diarrhea. In pregnant animals, the infection can also lead to abortions (Parida et al., 2015). The clinical manifestations may vary greatly depending on breed, age, immune status and virulence of the respective virus isolate (M. D. Baron et al., 2016; M. D. Baron, Parida, & Oura, 2011; Couacy-Hymann et al., 2007). Here, we explored further this variability by comparing the pathogenesis in goats of German breed of two PPRV isolates from India and the Middle East. One isolate was obtained from mountain gazelles that were affected by a PPR outbreak in the United Arab Emirates. Several thousands of mountain gazelles died while the neighboring sheep and goat population showed only few clinical signs. The other PPRV isolate originated from a mixed infection of goats in India by PPRV and FMDV. The infection was characterized by a mortality of 52 % and clinical signs characteristic for PPR and FMD infection. Due to the drastic socio-economic impact caused by PPR in developing countries, OIE and FAO launched a program to eliminate PPR globally by 2030. Effective vaccines and reliable diagnostic methods are required to achieve this goal (Albina et al., 2013; M. D. Baron et al., 2017; Jones et al., 2016; Santhamani, Singh, & Njeumi, 2016). There are numerous attempts to develop rapid and reliable tests for PPR diagnosis under field conditions (Ashraf et al., 2017; J. Baron et al., 2014; Li et al., 2018; Mahapatra et al., 2019). The objective is a simple test with little

additional laboratory equipment, which enables rapid results with adequate sensitivity and specificity (M. D. Baron et al., 2016; Howson et al., 2017). Therefore, we compared several commercially available rapid detection tests for PPRV. The LFD (Lateral Flow Device) developed at The Pirbright Institute (PESTE-Test) detects the PPRV H-protein with a published sensitivity of 84 % and specificity of 95 % for ocular and nasal swabs (J. Baron et al., 2014). The LFD from IDvet is based on N protein detection (ID Rapid® PPR Antigen, hereinafter referred to as ID Rapid) claiming a specificity of more than 99 % and a sensitivity of 100 % for eye swabs (ID.Vet, 2019a). As third antigen detection method for PPRV, the ELISA from IDvet (ID Screen® PPR Antigen Capture), which is a robust and well-established test in PPR diagnosis (Abraham & Berhan, 2001; Abubakar et al., 2011; Couacy-Hymann, Bodjo, Koffi, Kouakou, & Danho, 2009; Diop, Sarr, & Libeau, 2005; OIE, 2019), was also included in our study. This sandwich ELISA recognizes the PPRV nucleoprotein and is applicable to a range of samples such as ocular, nasal, mouth or fecal swabs, as well as tissue samples (Bataille et al., 2019; ID.Vet, 2019b). For validation as well as for calculation of sensitivities and specificities, we decided to use a PPRV-specific RT-qPCR assay because RT-qPCR is described to be more sensitive than virus titration (Couacy-Hymann et al., 2002; OIE, 2019). Furthermore, genome detection by PCR is the gold standard for confirmatory diagnosis of PPRV (Couacy-Hymann et al., 2009; Santhamani et al., 2016) and exhibits higher sensitivity than antigen ELISAs (Balamurugan et al., 2006; Couacy-Hymann et al., 2009). Here, we used the Polci-RT-qPCR assay targeting the nucleoprotein gene (Polci et al., 2015).

The aim of our study was to investigate the pathogenesis in goats of German breed of two PPRV isolates that showed different pathogenicity in the regional breed and use the samples obtained after experimental infection to compare distinct matrices including EDTA blood, ocular, nasal, mouth and fecal swabs for their suitability for rapid testing.

Materials and Methods

Ethics Statement

The animal trial was carried out in accordance with German legislation and approved by the competent authority State Office for Agriculture, Food Safety and Fisheries of Mecklenburg-Vorpommern (LALLF, Project license number: 7221.3-2-010/18). The animal trial was

performed in the biosafety level 4 experimental animal facilities at Friedrich-Loeffler-Institut (FLI), Insel Riems.

PPRV isolates

The PPRV isolates for the animal trialT were obtained from a PPR outbreak in Dubai, United Arab Emirates (UAE isolate), and from Shahjadpur, India (India isolate), respectively. The first isolate (SMRV/UAE/2018/V135/Dubai) was isolated from mountain gazelles (*Gazella gazella*) in August 2018 in which more than 5000 gazelles died near Dubai. Interestingly, domestic goats and sheep in the region of the outbreak showed only few clinical signs. A sheep and goat farm assumed to be the origin of the disease were tested and all goats proved to be seropositive for PPRV. More detailed information about the epidemiological situation in small ruminants at the time of the outbreak is not available. Thus, the virulence of this UAE isolate for native European goats was unclear. The second PPRV isolate (SMRV/IND/2013/V242.5/Shahjadpur) was obtained from a mixed infection of PPRV and FMDV in goats at Shahjadpur in India. Goats showed typical clinical signs such as high fever, mild lesions on gums and tongue, salivation and mucopurulent nasal discharge, dyspnea, diarrhea and swelling of the interdigital region. The mortality during this outbreak in February 2013 was 52 %. The animals of the affected farms were neither vaccinated against PPR nor against FMD (Kumar et al., 2016).
The PPRV UAE isolate was initially isolated at the Central Veterinary Research Laboratory in Dubai, including nine passages on Vero-Dog-SLAM (VDS) cells and subsequently passaged twice on VDS cells at the FLI. The PPRV-India isolate was passaged 15 times in co-cultured BHK21/Vero cells and three times in Vero cells (Kumar et al., 2016). For the animal trial, the isolate was further passaged six times on VDS cells at FLI.

Sequencing of PPRV isolates

RNA of cell-culture propagated PPRV was extracted with TRIzol™ LS (ThermoFisher Scientific, UK) and used in the cDNA synthesis system (Roche, Germany) for the generation of double stranded cDNA. The cDNA was submitted to Eurofins (Germany) for library preparation and highthroughput sequencing on an Illumina platform. Raw data were analysed with the Genious software package v11.1.5 (Biomatters, Ltd., New Zealand).
Animal trial and sample collection

The animal infection study was performed with two groups of animals, each with four male goats of German breed 'Deutsche Edelziege', aged 4 to 6 months. Animals were infected with the UAE isolate ($10^{4.38}$ $TCID_{50}$/ml) or the India isolate ($10^{5.75}$ $TCID_{50}$/ml). 2 ml of the inoculum was administered intra-nasally with a LMA™ MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex Medical, USA). Samples (EDTA blood, serum, ocular, nasal, mouth and fecal swabs) were collected at days -1, 3, 5, 7, 10, 12, 14, 17, 21, 28 post infection (dpi). In addition, fecal samples picked up from the stable floor for the individual animals were collected at irregular intervals. On -1 dpi pooled fecal samples were taken. Four days prior to infection, daily health checks of the animals including routine visual monitoring and measuring of the rectal temperature combined with clinical scoring (modified according to Pope et al. (2013), see Table S1 in supplementary materials) started. The experiment was terminated based on ethical end points for each individual animal, if the criteria according to Pope et al. (2013) were fulfilled. At the day of termination or at the final necropsy of the surviving animals (33 dpi), samples from lung, liver, spleen, mesenteric lymph node and mediastinal lymph node were collected and stored at -80 °C until further processing.

Processing of the organ samples

A panel of five tissue samples (lung, liver, spleen, mesenteric and mediastinal lymph nodes) was taken from all animals at the end of the study. All organs were homogenized in 800 µl cell culture medium without fetal calf serum using a 5 mm steel bead in the TissueLyser (Qiagen, Germany) and 100 µl homogenate were extracted with the NucleoMag® VET kit (Macherey-Nagel, Germany) on the KingFisher Flex automated extraction platform (ThermoFisher Scientific, UK). Extracted RNA was investigated according to a modified RT-qPCR assay of Polci et al. (2015). This assay was adapted to a reduced amount of master mix (10.0 µl) containing the reagents of the AgPath-ID™ One-Step RT-PCR kit (Thermo FisherScientific Inc., Waltham, USA) and 2.5 µl RNA template was added. PPRV-specific primers (PPR_Np-F298; PPR_Np-R366) and TaqManprobe (PPR_probe) were used as stated by (Polci et al., 2015) except that the probe used a FAM-Dye. The PCR reactions were run on a CFX96™ Real-Time PCR Detection System (BioRad Laboratories Inc., Hercules, USA) with the following temperature-time profile: 45°C for 10 min, 95°C for 10 min and 45 cycles at 95°C for 15 s, 56°C for 20 s and 72°C for 30 s.

Serological and molecular biological investigation of EDTA blood, serum, ocular, nasal, mouth and fecal swabs

For serological examinations the ID Screen® PPR Competition assay (IDvet, France) which contains recombinant PPRV nucleoprotein as antigen was used. Results were interpreted according to the manual: at S/N < 50 % samples were rated positive, at 50 % < S/N ≤ 60 % doubtful and at S/N > 60 % negative. The RNA extraction of EDTA blood and all swab samples was performed on the KingFisher Flex automated extraction platform (ThermoFisher Scientific, UK) using NucleoMag® VET kit (Macherey-Nagel, Germany). 100 µl of the homogenized sample were processed following the extraction manual and eluted in 100 µl of elution buffer. Subsequently, a PPRV-specific RT-qPCR targeted to the N gene of PPRV was performed for all samples (Polci et al., 2015) as described above.

Comparative validation of various rapid detection methods for PPRV

Three commercial antigen tests were compared: one antigen ELISA (ID Screen® PPR Antigen Capture) and two LFDs (PESTE-Test and ID Rapid). In our study, we evaluated the impact of the use of a common buffer (i.e. PBS) on the performance of IDvet test systems. Thus, three buffer systems for the sample collection were taken into account. For this purpose, each group of animals was divided into two subgroups of two animals each: the swabs of one subgroup were collected in a phosphate-buffered saline (PBS) standard buffer system (subgroup “standard”) and from the other subgroup with manufacturer-specific buffers (subgroup “IDvet/ Lillidale”). Mouth and fecal swabs of all animals and ocular and nasal swabs from the goats of subgroup “standard” (goat number G17, G20, G23 and G24) were processed in 2 ml of PBS. The ocular and nasal swabs from the goats of subgroup “IDvet/ Lillidale” (goat number G18, G19, G21, G22) were collected in manufacturer-specific buffers. For this purpose, two ocular and two nasal swabs were taken from each animal: The right eye swab was collected in 500 µl of ID Rapid - Elution Buffer (IDvet) and the left eye swab in 500 µl of buffer solution of the PESTE-Test (Lillidale Diagnostics).

A 5 % suspension of fecal samples was prepared according to the instructions of the manufacturer IDvet, France. For this, 5 g of feces were weighed into 1 ml of ID Rapid – Elution Buffer, vortexed and incubated for 5 minutes for the sedimentation of the homogenate. The supernatant was investigated by ID Rapid® PPR Antigen (IDvet, France), ID Screen® PPR Antigen Capture (IDvet, France) and the PPRV-specific RT-qPCR assay of Polci et al. (2015).

All LFDs were run according to the manufacturer´s protocols and assessed semi-quantitatively (- = negative; +/- = doubtful; + = weak positive; ++ = moderate positive; +++ = strong positive).

The ID Screen® PPR Antigen Capture ELISA was performed and evaluated according to the manufacturer´s instructions.

Sensitivities and specificities for the individual tests were calculated in comparison to the results of the reference method as described by (Parikh, Mathai, Parikh, Chandra Sekhar, & Thomas, 2008). Only samples with unambiguous results were used for the final calculation and samples which obtained the result "doubtful" were excluded. For the validation of the antigen detection methods we concentrated on samples derived from infected animals with limitations in sample number and volume. The extent of all positive samples used is limited to n = 82, whereof n = 67 were available for the validation of the ID Rapid and n = 15 for the PESTE-Test. Considering the negative samples, a panel of n = 29 was available, whereof n = 24 were used for the validation of the ID Rapid and n = 5 were used for the PESTE-Test (see results below).

Results

PPRV isolates used for the animal trial

Two PPRV isolates from an outbreak in goats in India and from diseased mountain gazelles in UAE were studied. The corresponding genomic sequences generated during this study are available under GenBank accession numbers MN369542 (UAE isolate) and MN369543 (India isolate). The raw sequencing data of both sequencing projects were submitted to the sequencing read archive (SRA) with reference PRJNA632993 (UAE strain) and PRJNA633015 (India strain). For the PPRV-India strain the nearly complete genome (coverage of 99.9%) could be generated with an average sequencing depth of 235. A substantial reduction (sequencing depth <30) of the sequencing depth must be ascertained for the last 20 nucleotide of the leader and trailer sequence of both termini. For the PPRV UAE strain the complete genome (100%) was sequenced with an average sequencing depth of 1324. Both isolates belonged to PPRV-lineage IV. Sequence analysis showed 98.8 % identity between the two isolates with overall differences in 190 nucleotides. Based on a BLAST analysis, both virus sequences have the highest homologies to several Indian PPRV isolates with an identity of more than 98 %.

Clinical manifestation and clinical score

The goats of both groups differed in the level of clinical manifestation. In the "India group", infected animals displayed low clinical scores. Only one goat developed elevated temperature

40.6 °C to 41.6 °C) on 3 to 5 dpi. In two other goats, mild nasal discharges were noted. Indeed, neither mucosal erosions nor any other severe clinical manifestations were observed (Fig.1). In contrast, the “UAE isolate” caused significantly more severe clinical signs, reaching peak scores on 10 dpi. All animals developed high fever (ranging from more than 40.0 °C to a maximum of 41.3 °C) beginning on 2 to 4 dpi and lasting for 6 to 8 days. The clinical signs after infection with the “UAE isolate” were mainly dominated by pyrexia for several days, accompanied by a deterioration of the general condition 3 to 7 days after the onset of fever characterized by inactivity, depression and loss of appetite. During the infection study all animals showed watery to mucosal-purulent nasal discharge, while facial mucosal lesions were not observed. During the clinical phase, all animals showed severe watery diarrhea. Two goats had to be removed on 10 dpi due to the severe clinical signs and the bad health condition. The two other animals had recovered completely by 18 dpi and 21 dpi, respectively (Fig.1).

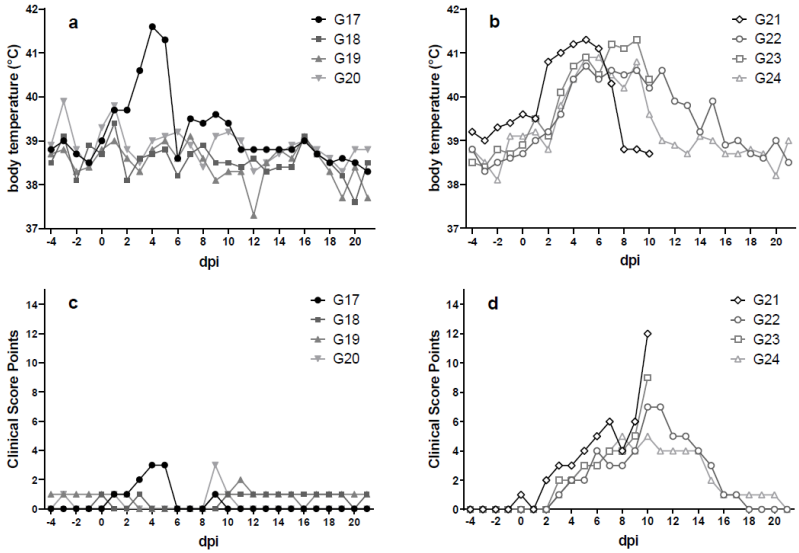

Figure 1 Clinical parameters of goats G17 to G24 during animal infection trial (−4 to 21 dpi): rectal body temperature for goat group 'India' (a) and goat group 'UAE' (b); average daily clinical score for goat group 'India' (c) and goat group 'UAE' (d)

RNA loads in EDTA blood and swabs

Fig. 2 shows the PPRV genome loads in EDTA blood and various secretions and excretions from animals of the two groups reflecting the different clinical manifestations. The "India group" reached maximal virus excretions on 7 dpi, except for nasal swabs where the maximum was secreted on 3 to 7 dpi. However, generally viral genome loads were limited with lowest Cq values of 25 to 26. Regarding continuity and maximal viral genome loads viral shedding was reflected best in nasal swabs, with nasal virus secretion lasting from 3 to 12 dpi. Ocular swabs were also suitable, but the detection rate was slightly decreased. Viral shedding via ocular fluid was detected from 3 to 12 dpi, with maximal Cq values of 30 to 31 on 7 dpi. EDTA blood and mouth swabs were inadequate exhibiting the lowest viral load similar to fecal swabs, with an intermittent shedding. In contrast, the viral genome load in EDTA blood and swabs from animals of the "UAE group" was substantially higher. Virus excretion started on 3 dpi

concomitant with the onset of clinical signs. At this time, nasal swabs yielded positive results for all animals, whereas the EDTA blood, ocular, mouth and fecal swabs of two out of four animals tested positive on 3 dpi. By 5 dpi all animals were positive in all sample matrices, except in EDTA blood, where three out of four animals were positive. Maximal virus shedding occurred on 7 to 10 dpi along with a subsequent elimination of two animals from the experiment. The highest viral loads were detected in ocular and nasal swabs with Cq values of 15 to 17 on 7 and 10 dpi. The highest viral loads in fecal swabs were detected on 7 dpi with Cq values of 22 to 26. Interestingly, in the fecal swabs a relatively constant viral genome load was detected after the viremia peak over the time. In contrast, a continuous decrease of the virus level was observed in blood, nasal and mouth swabs (Fig. 2).

RNA loads in organ samples

Five organ samples (lung, liver, spleen, mesenteric and mediastinal lymph nodes) taken on the day of necropsy were examined for viral RNA (Table 1). In the “India group” the mesenteric lymph nodes of all goats were weakly positive with Cq-values higher than 30. In addition, the spleen of one goat tested positive (G17). All animals were clinically inconspicuous at the time of necropsy, and showed no obvious pathological lesions. In contrast, the organ samples from group “UAE” showed higher RNA loads and the organs of animals removed prematurely from the experiment were all highly positive. The highest RNA loads were found in the mesenteric lymph nodes with maximum Cq-values of less than 20 in one goat (G21). In both convalescent animals, only the lymphatic organs were positive at the end of the trial (Table 1). Thus, lymphatic organs and in particular the mesenteric lymph nodes are recommended for a post mortem investigation of PPR suspicious cases.

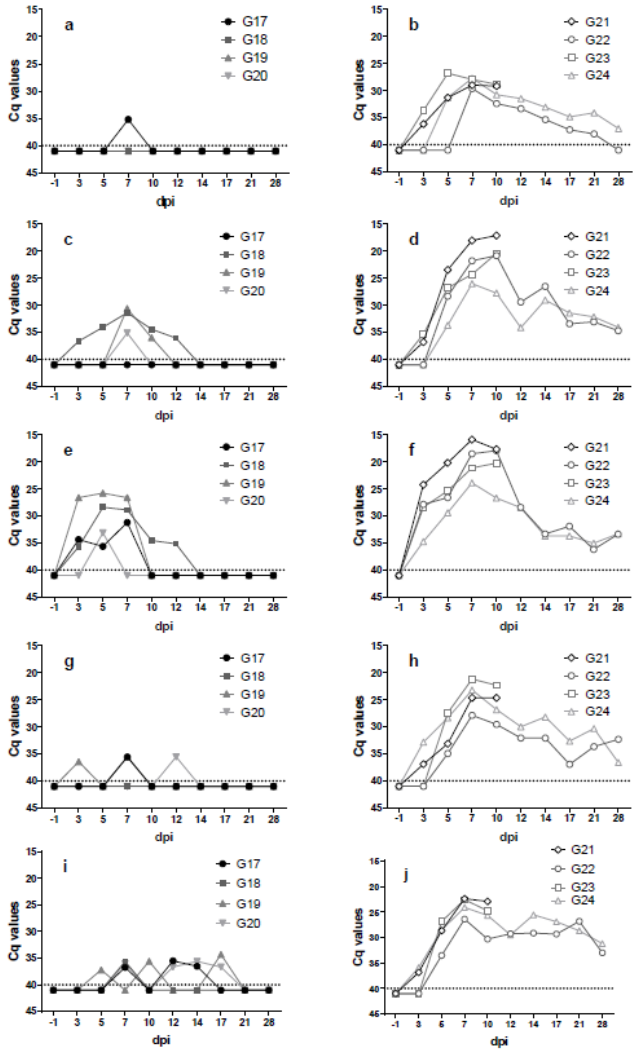

Figure 2 Loads of PPRV RNA in various sample matrices detected by RT-qPCR: left side (a, c, e, g, i) = 'India' group and right side (b, d, f, h, j) = 'UAE' group; EDTA blood = a + b; eye swabs = c + d; nasal swabs = e + f; mouth swabs = g + h; faecal swabs = i + j

Table 1 RNA loads (Cq values) from five organs examined with the RT-qPCR assay of Polci et al. (2015); G21 and G23 had to drop out of the animal trial on 10 dpi resulting in sampling of organ samples on the same day.

Group "India"	**G17**	**G18**	**G19**	**G20**
Lung	No Cq	No Cq	No Cq	No Cq
Liver	No Cq	No Cq	No Cq	No Cq
Spleen	36.90	No Cq	No Cq	No Cq
Lnn. Mesenteriales	35.69	36.86	36.89	33.30
Lnn. Mediastinales	No Cq	No Cq	No Cq	No Cq

Group "UAE"	**G21**	**G22**	**G23**	**G24**
Lung	27.32	No Cq	27.34	No Cq
Liver	27.88	No Cq	29.68	No Cq
Spleen	25.15	34.49	27.67	35.91
Lnn. Mesenteriales	19.96	28.35	26.47	33.10
Lnn. Mediastinales	24.50	32.10	29.14	34.11

Serological response to PPRV-infection

All eight infected goats seroconverted. An increase of anti-PPRV nucleoprotein antibodies was detected on 7 dpi for all animals. From 10 dpi all goats were seropositive until the end of the experiment on 33 dpi. Thus, the PPR-infection in both goat groups was confirmed (Fig. 3).

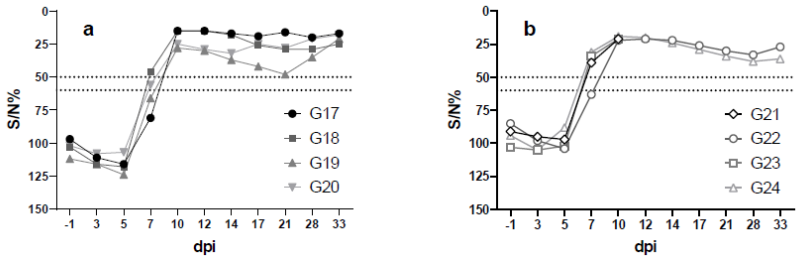

Figure 3 Serological results for goat group 'India' (a) and goat group 'UAE' (b) obtained with ID Screen® PPR Competition (IDvet, France)

Sensitivity and specificity of various antigen detection methods for PPRV

For comparative validation of PPRV detection methods, samples of goats from the group "UAE" were selected due to the greater range of viral secretion. RT-qPCR confirmed to be the most sensitive detection method and was used as reference for the evaluation of the antigen test systems (Table S2 to S5 in supplemental material). Both LFDs and the antigen ELISA achieved 100 % diagnostic specificity in all tested matrices and sample collection buffers. Using the ID-Rapid®-buffer, the detection rate of the antigen ELISA was 75 % for ocular and nasal swabs. The ID Rapid achieved similar data for both ocular and nasal swabs (71 % and 78 %, respectively). For the PESTE-Test nasal swabs proved to be the superior substrate with a sensitivity of 75 % compared to only 29 % for ocular swabs. For eye swabs, the ELISA (43 %) is better suited than the PESTE-Test (29 %).

The manufacturers of the antigen ELISA strongly recommend to use only the kit specific buffer systems as we performed it in our animal trial. Since animal trials require a relatively large volume of buffer for sample preparation and analysis, the use of commonly available buffers would facilitate the comparison between the test systems. The realization of equivalent test series with the PESTE-Test would have been desirable for the comprehensive presentation of the facts, but was not pursued by the study. To test the performance of common buffer systems like PBS as alternatives, ocular and nasal swabs taken in PBS were tested in parallel with the ID Rapid and ELISA (Table 2). The data show a drastic loss in sensitivity compared to the use of the manufacturer recommended buffers. Besides, we tested also the suitability of 5 % fecal

suspensions. Compared with the results of the RT-qPCR, the ELISA achieved a sensitivity of 59 % and the ID Rapid of 33 % (Table 2).

Table 2 Comparative validation of different rapid detection methods for SRMV compared to RT-qPCR (= No. tested); the data for the LFD and ELISA are given as "Number of samples tested positive (sensitivity in %)"

		ID Rapid®-buffer or PBS[†]			*Buffer of the PESTE-Test*[‡]		
	Buffer Solution	**No. tested**	**LFD**	**ELISA**	**No. tested**	**LFD**	**ELISA**
Positive samples							
Ocular swabs	specific[§]	7	5 (71.4 %)	5 (71.4 %)	7	2 (28.6 %)	3 (42.9 %)
	PBS	12	3 (25.0 %)	3 (25.0 %)	–	–	–
Nasal swabs	specific[§]	9	7 (77.8 %)	7 (77.8 %)	8	6 (75.0 %)	6 (75.0 %)
	PBS	12	3 (25.0 %)	3 (25.0 %)	–	–	–
Fecal samples	ID Rapid	27	9 (33.3 %)	16 (59.2 %)	–	–	–
Total	All	67	27 (40.3 %)	34 (50.7 %)	15	8 (53.3 %)	9 (60.0 %)
	corrected[¶]	16	**12 (75.0 %)**	**12 (75.0 %)**	15	**8 (53.3 %)**	
Negative samples							
Ocular swabs	specific[§]	4	0 (100 %)	0 (100 %)	3	0 (100 %)	0 (100 %)
	PBS	3	0 (100 %)	0 (100 %)	–	–	–
Nasal swabs	specific[§]	2	0 (100 %)	0 (100 %)	2	0 (100 %)	0 (100 %)
	PBS	2	0 (100 %)	0 (100 %)	–	–	–
Fecal samples	ID Rapid	13	0 (100 %)	0 (100 %)	–	–	–
Total		24	0 (100 %)	0 (100 %)	5	0 (100 %)	0 (100 %)

[†]swabs collected with the elution buffer of the IDvet kit or PBS were examined; [‡]swabs collected with the buffer solution of the Lillidale kit were examined; [§]buffer solution used is specific according to the manufacturer´s instructions of the LFD´s; [¶]considers only those samples that

have been taken in manufacturer-specific buffers and without fecal swabs to ensure comparability of the tests with each other.

Discussion

In this study, goats were infected with two PPRV isolates of different origin for pathogenesis studies. Samples of this animal trial were then used for the evaluation of various antigen detection methods (two LFDs and one antigen ELISA) in comparison to a PPRV specific RT-qPCR assay (Polci et al., 2015). Based on the available information about clinical signs and affected animal species from the outbreaks, we expected severe clinical symptoms of PPR in goats infected with the "India isolate" and only a mild form of the disease in goats inoculated with the "UAE isolate". The unexpected clinical course in the infection trials compared to the outbreak situations is most likely due to the different laboratory history of both virus isolates. Since the "India isolate" was passaged more frequently (especially on Vero cells), this may have led to attenuation (Balamurugan, Sen, Venkatesan, Bhanuprakash, & Singh, 2014; Eloiflin et al., 2019). On the other hand, the "UAE isolate" induced severe clinical signs in our naïve goats. No detailed information is available about the epidemiological situation of the domestic small ruminants in the region at the time of the massive PPR outbreak in wildlife, except for the farm assumed to be the origin of this outbreak where all goats were seropositive for PPRV. Goat and sheep farms in the vicinity of the semi-free ranging gazelles were in a distance of around 500 m. Subclinical infections or vaccination with attenuated PPRV could be relevant for the protection of the domestic goats and sheep (Balamurugan, Sen, et al., 2014). In addition, also the lack of direct contact between gazelles and domestic animals could be a reason for the clinically less-affected domestic herd of small ruminants (Anderson, 1995). Besides, the virulence of various PPRV isolates can vary depending on the goat breed and regional breeds may be less susceptible to PPRV isolates than naïve European goats which have never been in contact with PPRV.

For the "UAE isolate" the peak of viremia at 7-10 dpi was associated with massive clinical signs and very high RNA loads. Similar results have already been described for other PPRV isolates such as Côte d'Ivoire '89 (L I), Ghana/78 (L II), Kurdistan/2011 (L IV) and Morocco/2008 (L IV) (Parida et al., 2019; Pope et al., 2013; Wernike et al., 2014). Our findings confirm other studies because the highest RNA loads were also detected between 4 and 10 dpi (Parida et al., 2019;

Pope et al., 2013; Wernike et al., 2014). Here animals infected with the highly virulent "UAE isolate" seroconverted 4 to 7 days after the onset of the first clinical signs. Similar observations were published by Pope et al. (2013) and Wernike et al. (2014). In contrast, the "India isolate" only induced subclinical infection which paralleled the Cq values with a maximum of 25-26 in nasal swabs on 3 to 7 dpi. Our study aimed to identify differences regarding optimal sampling between sub-clinically and acutely infected animals. For both groups, PPRV-RNA was continuously detected only in nasal or ocular swabs. EDTA blood, mouth and fecal swabs proved to be less suitable for the detection of RNA in samples with a low viral load as observed in the subclinically infected animals because of the intermittent excretion. Regarding the comparison of sample materials in the "UAE" group, the RNA loads in EDTA blood, mouth and fecal swabs were higher than in the subclinically infected animals. Overall, our data demonstrate the preference for ocular and nasal swabs for genome as well as antigen detection of PPRV.

All antigen detection methods exhibited a specificity of 100 % in all tested biological matrices while the antigen ELISA provided superior or identical diagnostic sensitivity compared to the two LFDs. Based on the nasal and ocular swab samples the ID Rapid showed a slightly higher sensitivity compared with the PESTE-Test. In our tests, nasal swabs are the most suitable sample material for the three antigen detection methods tested in this study. The use of PBS for sample collection and dilution instead of the kit specific dilution buffer is not recommended because of the loss of sensitivity in the analyses.

Fecal samples are often discussed as suitable sample matrices due to non-invasive sampling for diagnosis of flocks as well as in screening programs for wild animal populations (Bataille et al., 2019). As part of our study, fecal samples were also tested in the LFD and in the antigen ELISA from IDvet. Similar to the analysis of ocular and nasal swabs test specificity is high also for fecal samples and the sensitivity seems to be sufficient for the testing of severely diseased animals. Therefore, further studies with a higher number of samples are necessary in the future, especially to provide more information about the diagnostic specificity of the different assays.

Based on previously published data (Wernike et al., 2014), we also examined several organs (lung, liver, spleen, mesenteric and mediastinal lymph node) for viral genome loads. The severely sick animals were positive in all five organs tested while those with subclinical disease were positive mainly in the mesenteric lymph nodes. The convalescent goats from the group "UAE" were clinically inconspicuous at the end of the experiment, but positive in the lymphatic organs examined.

For control and eventual global eradication of PPRV, rapid detection methods for use in field locations are crucial (M. D. Baron et al., 2017; Santhamani et al., 2016). They include a recently developed reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) and a real-time reverse transcription recombinase polymerase amplification (RPA) (Ashraf et al., 2017; Li et al., 2018; Mahapatra et al., 2019). We evaluated and validated three different antigen detection methods, ID Rapid® PPR Antigen (ID Rapid, IDvet, France), Rapid Field Test for PPRV Infection (PESTE-Test, Lillidale Diagnostics, UK) and ID Screen® PPR Antigen Capture ELISA (IDvet, France) in comparison with a RT-qPCR (Polci et al., 2015). The antigen ELISA and the ID Rapid-showed 75 % sensitivity for ocular and nasal swabs while the sensitivity of the PESTE-Test was 53 %. Samples taken in PBS showed a significant reduction in sensitivity (Table 2 and S4). As shown previously during the development of a LFD for the detection of foot-and-mouth disease virus, the buffer system for the stability of the target viruses may have a decisive influence on the performance of the test (Ferris et al., 2009). Buffers not recommended by the manufacturer must therefore be tested in advance for their suitability.

According to Jones and co-workers, the PESTE-Test was used in a Tanzanian study investigating PPR-suspected outbreaks of small ruminants. A total of 15 samples were tested and revealed a specificity of 100 % and a sensitivity of 54.5 % for ocular swabs (Jones et al., 2020). Thus, the PESTE-Test was more sensitive in the Tanzanian study than in our test series (28.6 %) concerning ocular swabs. In contrast, the PESTE-Test seems to be more suitable when using nasal swabs (75.0 %) The different results may be explained by different sampling strategies because in the Tanzanian study, clinically ill animals were sampled for the confirmation of PPRV antigen. In contrast, in our infection study, low-loaded and high-loaded samples were collected in the course of an infection to validate the diagnostic performance of the LFDs. LFDs are wellsuited for acutely diseased animals, but show weaknesses in low-loaded animals (subclinically diseased goats or samples from a later stage of infection). According results from the Tanzanian study confirm our findings.

To analyze suitability of the antigen detection systems as pen-side test, they were evaluated using relevant criteria as shown in Table 3. The ID Rapid performed well in this regard because of its simple handling, feasible sensitivities and the ability to store the kit at room temperature without special reagent preparations. Results are available within 30 minutes which is a strong advantage for diagnostics in the field. For individual samples results were weakly positive (doubtful) and such result can be questionable especially under field conditions. This was seen in both LFDs, although it was more common in the PESTE-Test (Table S1 to S4). The advantages

of PESTE-Test are its simple handling, results are also available within 30 minutes and no additional laboratory equipment is needed. The supplied swabs in the PESTE-Test proved to be unsuitable as ocular swabs for goats. The test is unfavorable as pen-side test because the kit has to be stored at refrigerator temperature. The antigen ELISA takes about two hours for skilled users but it provides best results in terms of sensitivity and produces unambiguous results. The need for additional laboratory equipment such as small ELISA reader, pipettes and plastic material, its relative long runtime and its storage requirements makes this test not very suitable for the field. In our study design, the ELISA provided valuable comparative data for the evaluation of the LFD tests because it is also based on antigen detection. Antigen detection systems show advantages and disadvantages as for their suitability as a pen-side test (Table 3). The method of choice has to be determined individually according to the application requirements, the objective and the time required. The data presented here should support the necessary decisions. Nevertheless, based on the excellent diagnostic specificity of the rapid tests, positive results can be evaluated as “true”. In contrast, negative test results can be justified on the reduced analytical sensitivity of the rapid antigen tests and must be treated with caution, especially in subclinical infections.

Table 3 Comparative evaluation of various rapid detection methods for SRMV with regard to the suitability as a pen-side test

	Lateral Flow Devices		*Antigen ELISA*
	ID Rapid® PPR Antigen	**PESTE-Test**	**ID Screen® PPR Antigen Capture**
Simplicity	++	+++	+[a]
Good runnability of the test strips	+++	+	n.a.
Duration (without preparation time)	30 min	30 min	2 hours
Simple instructions for use	++	+++	+[a]
Suitability of the swabs	+++	+	n.a.
Additional equipment necessary	No	No	Yes
Preparation of reagents required			
Reagents/ device should be brought to ambient temperature	No	Yes	Yes
Reagents have to be mixed in advance	No	No	Yes
Room temperature (18°C to 30°C)	+++	–	–
Refrigerator temperature (2°C to 8°C)	+	+++	+++
Sensitivity	75.0 %	53.3 %	75.0 %
No doubtful results	++	+	+++
Cut-off (in Cq-values)	25 - 26	24 – 26	24 – 26
Test system applicable for differential diagnosis	No	No	No

+++ = completely agree; ++ = rather agree; + = is insufficient; – = strongly disagree; n.a. = not applicable; [a]for experienced users only

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Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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V DISCUSSION

Various degrees in the clinical manifestation of different PPRV isolates

Only one serotype is known for each representative of the genus *morbillivirus*, however, PPRV is further classified into four distinct genetic lineages. Phylogenetic data revealed that lineage IV is currently the most abundant cluster of PPRV (Parida et al., 2016), containing also the two isolates we examined here in our studies.

The clinical signs we observed in goats of German breed were contrary to the ones described in the associated outbreaks, and between the two isolates available for our studies. Originally, the India isolate (strain: SMRV/IND/2013/V242.5/Shahjadpur; accession number in GenBank: MN369543) was involved in mixed infections of FMDV and PPRV in goats in the Indian region Shahjadpur with clinical signs such as high fever, lesions in the oral cavity, nasal and oral discharge, dyspnoea, diarrhoea and a mortality rate of 52 % (Kumar et al., 2016). Contrary, the UAE isolate (strain: SMRV/UAE/2018/V135/Dubai; accession number in GenBank: MN369542) originally affected mountain gazelles (*Gazella gazella*). In August 2018, more than 5000 gazelles died while the small ruminants of the vicinity showed less indication concerning a PPRV infection.

In our pathogenesis studies, four goats per group were infected intranasally with these two PPRV isolates. The goats of the India group showed only slight clinical signs including high temperature for three days in one goat and mild nasal discharges in two other goats. In the UAE group, all animals developed pyrexia for 4 to 6 days accompanied by inactivity, depression and loss of appetite. All goats showed watery to mucosal-purulent nasal discharges and severe watery diarrhoea. According to the severe clinical signs, two goats were removed from the UAE group. The goats of both groups were seropositive from 10 dpi on up to the end of the experiment (33 dpi). In conclusion, the goats of the India group underwent a subclinical infection while the goats infected with the UAE isolate showed an acute PPRV infection. In addition, a defined panel of organ samples (lung, liver, spleen, mesenteric and mediastinal lymph nodes) was examined with respect to the RNA loads. As expected, the mesenteric lymph nodes of all eight goats were enlarged and positive for PPRV-RNA. Besides, positive results were also obtained in the spleen and the mediastinal lymph nodes for both goats of the UAE group

that completely recovered from infection. Furthermore, both goats of the UAE group that had to be removed were RNA-positive in all organ samples taken.

Up to now, several animal trials were carried out studying the pathogenesis of different PPRV isolates (Côte d'Ivoire ´89 (LI); Bissau Guinea (LI); Guinea Conakry (LI); Ghana/78 (LII); Nigeria 75/1 (LII); Sudan Senner (LIII); India Calcutta (LIV); Kurdistan/2011 (LIV); Morocco/2008 (LIV) for instance). All studies aimed to investigate several aspects such as the degree in clinical manifestation, incubation periods, mean survival times, date of death, mortality rates, virus shedding in various sample materials, viral RNA loads in different tissues and virulence factors, respectively (Couacy-Hymann et al., 2007a; Enchery et al., 2019; Parida et al., 2019; Pope et al., 2013; Wernike et al., 2014). The spectrum of clinical signs that was stated in our goats of German breed coincide with those signs described for PPR disease in outbreaks as well as in animal infection studies (Diop et al., 2005; Enchery et al., 2019; Hammouchi et al., 2012; Pope et al., 2013; Wasee Ullah et al., 2016; Wernike et al., 2014).

The first clinical sign observed in our goats was an increase in body temperature and appeared 2 to 4 dpi. Nasal discharges started on 7 to 10 dpi and the alteration of fecal consistency on 5 to 9 dpi. Couacy-Hymann and co-authors approved our findings when investigating six different PPRV isolates (Côte d'Ivoire/89; Guinea Conakry; Bissau Guinea; Nigeria 75/1; Sudan Sennar; India Calcutta) in goats. Initially, their goats developed also pyrexia on 2 to 5 dpi. As in our studies, further clinical signs appeared after the increase of body temperature, namely nasal discharges on 4 to 7 dpi and diarrhoea on 4 to 9 dpi (Couacy-Hymann et al., 2007a).

In our India group, all animals survived until the end of the study while in the UAE group two goats had to be removed on 10 dpi and the other two recovered from an acute PPRV infection until the end of the pathogenesis study. Considering the results from Couacy-Hymann and co-authors, the date of death varies from 7 to 12 dpi and the mean survival time is indicated with 9 to 10.2 days (Couacy-Hymann et al., 2007a) while seven out of 30 goats (23.3 %) recovered from the infection. Comparing these data with the results of Wernike and co-authors examining the PPRV isolate Kurdistan/2011 (LIV), the day of removal for all three inoculated goats were between 7 to 12 dpi confirming the afore mentioned analyses (Wernike et al., 2014). Besides, Couacy-Hymann and co-authors reported mortality rates ranging from 0 % (Nigeria 75/1) to 100 % depending on the lineage of the PPRV isolate used.

The results obtained from our pathogenesis studies with two different PPRV isolates showed a varying degree in the clinical manifestation in goats of German breed while all animals became seropositive. Thus, the results indicate an influence of breed (e.g. regional versus German breeds) and host species (e.g. gazelles versus goats) on the virulence of PPRV isolates. Furthermore, the immune status of regional breeds e.g. due to vaccination or the endemic situation has to be taken in consideration.

Besides those factors, both isolates were also processed differently. In fact, the Indian isolate was passaged 24 times in co-cultured BHK21/ Vero cells, Vero cells and VDS, respectively and the UAE isolate was passaged 11 times on VDS. These findings might also indicate an influence of in vitro virus processing (number of passages, types of cell lines used) which could result in attenuation of viral strains and hence leading to different clinical manifestations in the animals as it was already described by Enchery et al., 2019.

The selection and also a more detailed description of the animals used for infection studies should receive more attention with regard to age, gender and breed of the animals, since the information related to this properties are not always available (Bataille et al., 2019; Couacy-Hymann et al., 2007a; Wernike et al., 2014). Especially, the influencing factor of regional breeds is often described in the context of PPRV infections (Couacy-Hymann et al., 2007a; Diop et al., 2005; Enchery et al., 2019) and is, as in our study, often a matter of discussion when clinical signs in experimentally infected goats differ from the signs observed in the originally affected animals during an outbreak. Thus, comparative pathogenesis studies of different breeds (e.g. German versus African breeds) can strengthen these hypotheses. In this context, also the immunological status of PPRV-infected goats is of great interest.

As shown by Parida et al. 2019, goats that were infected with two different PPRV isolates of lineage II (Ghana/78) and IV (Morocco/2008), respectively, showed e.g. a depression in white blood cells during the course of an infection suggesting that the immunological status should be given more consideration for further studies with PPRV-infected animals. In addition, a case report provides first indications of a differing susceptibility of female in contrast to male animals and also a varying degree in clinical manifestation regarding the age of the goats affected (Soundararajan et al., 2006).

For an extended knowledge concerning the pathogenesis and epidemiological interaction of PPRV in wildlife (particularly suidae, bovinae, camelidae), future studies should target even a wider range of host species (Rahman et al., 2020). Profound epidemiological data are also necessary to assess the relevance of wildlife species. In particular, the transmission dynamics on the interface between wildlife and livestock, maintenance of PPRV in wildlife without domestic hosts, and retransfer to livestock is not clearly understood, but more detailed knowledge is necessary to control the disease effectively (Bataille et al., 2019; Fine et al., 2020). Concerning the global eradication campaign, launched by the OIE and the FAO, wildlife populations have to be included in the implementation of control and surveillance strategies (Couacy-Hymann et al., 2005). Moreover, a profound knowledge on all susceptible hosts (domestic and wild species) and the availability of reliable detection tools for both domestic and wildlife species that are suitable for control programmes and sero-surveillance should be strived (Fine et al., 2020).

Evaluation of various sample materials with regard to the level of virus excretion in the course of an infection with PPRV

In our *in vivo* studies, EDTA blood, ocular, nasal, mouth and faecal swabs were investigated according to their viral loads in terms of nucleic acid and antigen content in order to determine the most appropriate sample matrices for diagnostic purposes in the field. As shown in our studies, RNA loads vary depending on the sample material selected. According to the various degrees of clinical manifestations in both goat groups, our tests also stated a varying amount of viral shedding between subclinically and acutely infected goats. Hence, the samples of the UAE group contained high viral loads (high-loaded) and the samples of the India group in contrast low viral loads (low-loaded). This range of viral loads allowed the generation of very valuable reference material panels with different antigen, virus and genome loads.

Assessing our results, the highest amounts of PPRV RNA loads were detected in ocular and nasal swabs compared to EDTA blood, oral and faecal swabs. Both, eye and nasal swabs were PCR-positive for a long period in the course of the experimental infection. The RNA detection window for high-loaded nasal and ocular swabs is set between 3 to 28 dpi. In contrast, for low-

loaded sample materials, the detection window is much narrower as it ranges between 3 to 12 dpi for nasal and ocular swabs.

Summarizing our diagnostic validation results, nasal swabs are the sampling material of choice for a reliable diagnosis of PPRV based on either antigen or molecular detection. It remains to be considered that a correct molecular diagnosis of acutely infected animals is feasible within the detection window, also when EDTA blood, ocular, mouth or faecal swabs are used. However, during the incubation period of acutely diseased animals, RNA detection is already feasible before the first clinical signs appear, thus being a "diagnostic time window" in naturally infected animals that can be overlooked (Couacy-Hymann et al., 2007b). In contrast, the RNA detection in the low-loaded samples is intermittent, especially from EDTA blood, oral and fecal swabs, and the detection window is much smaller complicating a correct and timely diagnosis. Overall, the reliable molecular detection of PPRV of subclinically infected animals remains challenging because these animals are not clinically diseased and diagnostic samples as well as the time point of sampling has to be chosen very carefully. Otherwise, reliable PPRV diagnosis of subclinically infected animals could also be expanded by the additional detection of antibodies.

Parida and co-authors recommend the sampling of nasals swabs or paired sample analysis consisting of nasal samples and EDTA blood for the molecular diagnosis of PPRV (Parida et al., 2019). The results that were obtained in my study confirmed also nasal swabs as the sample material of choice for the detection of PPRV genomes. Nevertheless, according to our results, there is no evidence that EDTA blood is the most appropriate sample material which is also consistent with the data published by Enchery and co-authors (Enchery et al., 2019). In the latter reference, PPRV RNA amounts were higher in ocular swabs than in EDTA blood inducing the authors to recommend also ocular swabs for the detection of PPRV RNA. In addition, Couacy-Hymann and co-authors stated that ocular swabs are slightly more sensitive than nasal swabs (Couacy-Hymann et al., 2009) which is in contrast to the results we obtained and should be therefore investigated in more detail in future studies.

In comparison to the real-time RT-PCR-based RNA detection, the same panel of samples was also tested with a commercially available antigen ELISA (ID Screen® PPR Antigen Capture). The detection window for the antigen detection of high-loaded samples was stated between 3 to 14 dpi for nasal swabs and between 5 to 10 dpi for ocular swabs. In comparison to the data

based on RT-qPCR, the detection window for antigen detection must be defined more narrowly, thus restricting the PPRV diagnosis based on the antigen ELISA. Notably, a direct correlation between RNA loads and the OD values of the antigen ELISA could not be observed. Nevertheless, antigen detection methods can be justified as POC testing, considering the use of LFDs on the pen-side for livestock or for testing wildlife animals directly in the field that enables an advanced diagnosis of strong positive samples (see below).

The results of diagnostic test systems are influenced not only by the kind of sample material itself but also by the conditions of the sample materials used (freshness at the time of examination, storage conditions, transport under adequate conditions, decomposed material). For the detection of PPRV, fresh material is strictly recommended to ensure a good quality of viral RNA (Bataille et al., 2019; Borsanyiova et al., 2018; Howson et al., 2018), and because of the rapid inactivation of PPRV in the environment (Cameron, 2019; Kumar et al., 2014; Mahapatra et al., 2019) and in dead animals (Couacy-Hymann et al., 2007b). Besides, several attempts were made to ensure the quality of the sample materials even when handled under unfavourable transport and storage conditions such as to use alternative transport materials (filter paper cards so-called FTA® cards or LFDs), the usage of stabilizing buffer systems or viral recovery devices based on the drying of liquid sample materials under ambient temperatures (Barr et al., 2013; Fowler et al., 2014; Sakai et al., 2015). Otherwise, a cooling chain for the storage of fresh samples has to be guaranteed if the samples cannot be investigated immediately. But multiple freeze-thawing processes in the laboratory can also hamper the quality of samples and the yield of viral RNA (Couacy-Hymann et al., 2007a).

Moreover, the results of the faecal swabs should be considered separately regarding their opportunity to adapt the sampling method to a non-invasive tool and thus, simplify the sampling of wildlife species or large flocks (Bataille et al., 2019; Wasee Ullah et al., 2016). For the collection of faecal samples neither capturing nor the handling of the target species is needed. However, faecal samples contain inhibitor substances that influence the results of PCR and ELISA assays negatively (Bataille et al., 2019; Wilde, Eiden, and Yolken, 1990). Indeed, in our studies faecal swabs showed lower PPRV genome loads compared to nasal and mouth swabs. For high-loaded samples, RNA detection was continuously feasible from 3 to 28 dpi while for low-loaded samples, the RNA in faecal swabs was detected intermittently between 5 to 17 dpi. Compared to recent studies of Bataille and co-authors, the diagnostic window for the detection of PPRV genomes in faecal swabs was limited to 5 - 14 dpi (Bataille et al., 2019). Our

studies confirm those data and show that PPRV genomes can be detected up to 28 dpi in acutely diseased animals in these sample materials. Besides, Bataille and co-authors also examined faecal material with an antigen ELISA indicating a detection window lasting from 7 up to 14 dpi and an intermittent positive antigen detection in the later stages of an infection.

Furthermore, efforts are also made to detect PPRV specific antibodies in faecal samples (Bataille et al., 2019), but additional studies are needed to optimize the protocols for antibody detection in faecal samples. In conclusion, the results indicate that faecal materials are a justifiable sampling material for genome detection in acutely diseased animals with sufficient virus replication and shedding. Moreover, in subclinically infected animals, the protocols focussing on testing faecal material (nucleic acids, antigen or antibodies) should be further adapted or results should be confirmed by additional diagnostic tools.

Another possibility to bypass the handling of animals for diagnostic purposes and an opportunity to carry out herd-wise investigations are bulk milk samples. First evidence is provided that female goats excrete PPRV in milk (Clarke et al., 2018). Further reliable data are needed concerning the start point and duration of viral excretion in milk, amounts of virus shedding in the course of an infection, and suitable detection targets (virus, antigens, nucleic acids, antibodies). Besides, it has to be verified whether diagnostic detection methods can be adapted to the sample matrix milk in terms of diagnostic performance (Reid et al., 2006). Due to the non-invasive and simple sampling of milk, screening programmes using bulk milk samples have been implemented for further viruses (Drew, Yapp, and Paton, 1999; Reid et al., 2006). Bulk milk sampling should be considered for PPRV surveillance, thus the proof of its feasibility has to be done in future studies. In this context, milk as infection route for offspring and also between animals was discussed (Clarke et al., 2018), but the protection of offspring via colostrum uptake after the birth should also considered (Balamurugan et al., 2012b; Diallo, 2006).

Considering the outcome of our studies, the different diagnostic requirements for acute and subclinically diseased animals were precisely pointed out and thus, deliver valuable upgrades according to previously studies which are also focusing on optimal sampling strategies and sampling time points. For reliable PPRV diagnosis based on molecular detection, some well-suited methods of sampling are available, such as the use of ocular and nasal swabs (Enchery et al., 2019; Parida et al., 2019). As confirmed in our studies, nasal swabs are the sample

material of choice in terms of RNA detection, providing reliable results in acutely diseased animals. However, in subclinically diseased animals, some diagnostic restrictions have to be accepted. Regarding the sampling, swabs are less invasive than the collection of blood or serum (Parida et al., 2019), but the animals must be caught and manipulated to get this sample material as well. Further options that overcome the drawbacks of manipulating animals for diagnostic procedures are the collection of faecal samples and the use of bulk milk samples. Both faecal and milk samples are more elaborate in sample preparing regarding the selection of extraction methods and the adaption of PCR and ELISA assays (Bataille et al., 2019; Reid et al., 2006). Thus, comparative studies should be conducted in the future that weigh up various forms of non-invasive sampling (e.g. faecal versus milk samples) in terms of their diagnostic performance (sensitivity, specificity, reliability, detection window) and their practical benefit (duration, financial effort, workload) for POC diagnosis or surveillance programmes. The type of detection bodies should also be considered when assessing diagnostic methods, because RNA loads in samples are assumed to be correlated with the shedding of virus (Couacy-Hymann et al., 2009). Indeed the positive proof of RNA is not necessarily accompanied with the excretion of infectious virus, especially in the later stages of an infection (Enchery et al., 2019).

Development, optimization and validation of PPRV-rapid tests according to their diagnostic performance and their suitability for pen-side/point-of-care diagnostics

Several antigen detection methods were comparatively evaluated according to their diagnostic performance as well as to their features for diagnostic testing in the field. For the antigen detection of PPRV, two LFD test kits (ID Rapid® PPR Antigen from IDvet, and the PESTE-Test from Lillydale) and one ELISA (ID Screen® PPR Antigen Capture) are commercially available. As ocular and nasal swabs are a less invasive sampling method, containing also the highest amounts of PPRV genomes, the three antigen detection methods were tested with a panel of ocular and nasal swabs collected from our pathogenesis studies. Faecal samples were tested with the ID Rapid only.

Best results in terms of diagnostic sensitivity were demonstrated for both the antigen ELISA and the ID Rapid LFD. For both tests, the sensitivity determined with the ocular and nasal swabs was 75 %. In contrast, the PESTE-Test achieved a markedly lower sensitivity of 53.3 %. In

addition, the faecal samples (33.3 %) were less sensitive than the preferred ocular or nasal swabs (75 %) in their application for the ID Rapid. The specificity for all diagnostic tests and all sample materials was 100 %. Concerning their diagnostic reliability in general, LFDs exhibit a good specificity but are less sensitive (Mashayekhi et al., 2010; Tang et al., 2016; Zhang et al., 2019) which was confirmed and specified by the results of my thesis.

Previous references also indicate that the sensitivity of antigen LFDs for further human and animal viruses range from 64 % to 94.6 %, while the specificity of the same LFDs is in most cases more than 97 % (Ferris et al., 2012; Ferris et al., 2010a; Ferris et al., 2010b; Ferris et al., 2009; Jiang et al., 2011; Sambandam et al., 2017; Yang et al., 2019; Zhang et al., 2019).

In agreement with previous references, LFDs perform in a reliable matter in terms of samples containing high amounts of the virus (Howson et al., 2017). These findings indicate that positive results delivered by those LFD-based antigen assays are reliable, but negative results have to be treated with caution and should be confirmed with more sensitive methods. However, our studies also demonstrated that antigen LFDs exhibit a comparable sensitivity compared to antigen ELISAs which is in line with findings of Howson et al., 2017b. Moreover, the LFDs delivered results within only 30 minutes while the antigen ELISA required a quadruple of time. LFDs are rapid, easy-to-use, small-sized and portable diagnostic tools and thus, being a relevant option for pen-side/POC diagnostics. An additional benefit to facilitate the application in the field is the storage possibility of several LFD kits at room temperature. Considering all strengths and weaknesses of LFDs, they are suitable as diagnostic pre-tests in acutely diseased animals delivering results in a short time and thus, providing first decision-making aids on the POC. In conclusion, a reasonable combination out of upstreamed, cost-extensive LFDs and downstreamed, rapid molecular diagnostic methods are a feasible diagnostic option for extensive investigations in the field.

Considering the pros and cons of the commercially available POC diagnostic tools for PPRV described above, a further study (data are currently under submission) has focused on the development of a field-ready molecular diagnostic test for PPRV aiming to optimize the POC tests in resource-limited settings (sensitive, specific, user-friendly, time-saving, portable). The idea of the new RT-qPCR test system focuses on the simultaneous detection of PPRV and further pathogens of field samples in less than one hour including all work steps from the sample preparation to the result. The design of the fast molecular test is created as rapid

extraction plus a high-speed RT-qPCR approach detecting five pathogens (PPRV, FMDV, parapoxvirus ovis, GTPV, Mccp) in parallel. The high-speed RT-qPCR approach lasts only around 35 minutes indicating a good turnaround time for a nucleic acid detection tool and the fast RNA nucleic extraction lasts approximately seven minutes. The design as modular test system simplifies the adaptation towards changing conditions in the field (anamnesis, suspected diagnosis, the endemic situation), client requests (diagnosis of a single herd, outbreak investigations, epidemiological purposes) or individual requirements (number of samples, differential diagnosis aimed, selection of internal controls) as well as easing the integration of internal controls. For simplifying the new test system as much as possible, mouth swabs are preferred and the test was implemented with a lyophilized PCR kit, thus being portable at ambient temperature. For the use at the POC, a “mobile laboratory” consisting of a robust extraction platform and a portable RT-qPCR cycler have to be implemented. As mentioned above, a combination of the simple and cheap LFDs and the new molecular POC test system seems feasible and will further optimize the possibilities for PPRV diagnostics. Finally, the reference sample panel described in my study will help to optimize and validate future test systems, such as these molecular POC test systems.

VI SUMMARY

Peste des petits ruminants (PPR) is a highly contagious disease that is increasingly spreading across the African and Asian continent and has recently crossed the European borders. Main hosts are small ruminants but there is a plenty of references according to which the potentially susceptible host species have to be expanded. Concerning the elimination of this disease up to 2030, strived by FAO and OIE, reliable diagnostic tools, improved vaccines and a better knowledge about the disease are necessary. In this context, the here reported study aimed to characterize various PPR virus (PPRV) isolates *in vivo* and to improve diagnostic tools detecting PPRV in a rapid and reliable way, ready-to-use in the field. Therefore, groups of four goats of German breed were intranasally infected with two distinct PPRV isolates. One PPRV strain was originally isolated from goats showing clinical signs of a mixed infection of PPRV and FMDV in the Indian region Shahjadpur ("India isolate"; strain: SMRV/IND/2013/V242.5/Shahjadpur; accession number in GenBank: MN369543). The other PPRV isolate was involved in a severe outbreak near Dubai, UAE, causing high mortalities in mountain gazelles ("UAE isolate"; strain: SMRV/UAE/2018/V135/Dubai; accession number in GenBank: MN369542). The so-called "India isolate" caused mild symptoms in our goats of German breed while the goats infected with the "UAE isolate" showed severe clinical signs of a PPRV infection (e.g. pyrexia, deterioration of the general condition, mucosal-purulent nasal discharge, watery diarrhea). All animals were tested positive for antibodies against PPRV. While the goats of the India group underwent a subclinical infection, the goats of the UAE group developed a severe acute disease. Thus, the collection of samples containing low and high viral loads depending on the course of an infection in the goats was possible, and the sample panel was used to select the most suitable sample matrices for PPRV detection. Several sample materials (EDTA blood, ocular, nasal, mouth and fecal swabs) were collected and tested for their amounts of viral RNA by a PPRV-specific RT-qPCR. The highest amounts of PPRV genomes were detected in nasal swabs followed by mouth swabs suggesting both matrices as suitable sample materials for diagnostic purposes. In addition, three antigen detection methods were evaluated regarding their diagnostic reliability and suitability for pen-side/point-of-care (POC) testing. For this purpose, we analysed one antigen ELISA (ID Screen® PPR Antigen Capture from IDvet) and two assays based on the lateral flow device (LFD) technology (ID Rapid® PPR Antigen from IDvet, and PESTE-Test from Lillydale).

Generally, nasal and mouth swabs showed better results in their diagnostic performance compared to fecal samples. The antigen ELISA and the LFD-ID Rapid performed best in our studies, each with a sensitivity of 75.0 %. In contrast, the PESTE-Test achieved a sensitivity of only 53.3 %. Concerning the practical point of view, the tested LFDs delivered results within 30 minutes towards less than two hours for the antigen ELISA. The LFDs are also very simple in their handling and need no further laboratory equipment. Another strength of the ID Rapid is e.g. its storage capability at room temperature that considerably simplifies its diagnostic use in the field (especially in African or Asian countries).

Depending on the individual requirements, either for routine diagnostics, comprehensive epidemiology studies or for POC diagnostics during an outbreak, different rapid test systems are available for the detection of PPRV, some of which have been tested comparatively in our studies. In terms of laboratory equipment, user skills, diagnostic performance requirements (sensitivities, specificities, time requirements), clinical course of infection in the animal (expected low-load versus high load samples), and individual objectives, the tests validated in our study have their value for the detection of PPRV. Nevertheless, the results presented here should be mainly understood as decision support for the end user in choosing the appropriate diagnostic test. And the reference sample panel described in my study will help to optimize and validate future test systems, such as molecular POC test systems.

VII ZUSAMMENFASSUNG

Die Pest der kleinen Wiederkäuer (PPR) ist eine hochkontagiöse Erkrankung, die sich zunehmend über den afrikanischen und asiatischen Kontinent ausgebreitet und nun auch die europäischen Grenzen übersprungen hat. Die Hauptwirte sind kleine Wiederkäuer, jedoch gibt es eine Vielzahl von wissenschaftlichen Abhandlungen, nach denen die potentiell empfänglichen Wirtstiere erweitert werden sollten. In Bezug auf die Eradikation der Erkrankung im Jahr 2030, die seitens der FAO und OIE angestrebt wird, sind verlässliche diagnostische Methoden ebenso wie sichere Impfstoffe sowie ein besseres Verständnis zur Erkrankung erforderlich. Vor diesem Hintergrund war meine Arbeit darauf ausgerichtet, Pathogenesestudien mit verschiedenen PPR Virusisolaten durchzuführen und Diagnostiktools für eine schnelle sowie verlässliche Detektion von PPR-Viren (PPRV) im Feld zu testen und zu verbessern. Pro Gruppe wurden vier Ziegen deutscher Herkunft intranasal mit zwei verschiedenen PPRV-Isolaten infiziert. Einer dieser PPRV-Stämme (“India Isolat”; Stamm SMRV/IND/2013/V242.5/Shahjadpur) wurde ursprünglich aus Ziegen der indischen Region Shahjadpur isoliert, welche klinische Symptome zeigten, während das andere PPRV-Isolat (“UAE Isolat”; Stamm SMRV/UAE/2018/V135/Dubai) einen schweren Ausbruch mit hohen Mortalitäten in Echtgazellen (*Gazelle gazella*) nahe Dubai, UAE, verursachte. Das so gennannte „India Isolat“ verursachte in den Ziegen deutscher Herkunft milde Symptome, während die Ziegen, die mit dem „UAE Isolat“ infiziert wurden, schwere klinische Symptome einer PPRV-Infektion (hohes Fieber, Verschlechterung des Allgemeinzustandes, muco-purulenter Nasenausfluss, wässriger Durchfall) zeigten. Alle Tiere wurden positiv auf Antikörper gegen PPRV getestet. Die Ziegen der Indien-Gruppe zeigten einen subklinischen Krankheitsverlauf, während die Ziegen der UAE Gruppe eine akute Infektion zeigten. Somit war die Probennahme von Materialien mit niedrigem und hohen Viruslasten in Abhängigkeit von den unterschiedlichen Infektionsverläufen in den Ziegen möglich, was für die Auswahl des am besten geeigneten Probenmaterials für die Detektion von PPRV genutzt wurde. Es wurden unterschiedliche Probenmaterialien (EDTA-Blut, Augen-, Nasen-, Maul- und Kottupfer) auf ihren Gehalt an PPRV-Genomen getestet, die mittels PPRV-spezifischer RT-qPCR detektiert wurden. Der höchste Gehalt an Nukleinsäure des PPRV wurde in Nasentupfern gefolgt von Maultupfern detektiert, sodass beide Matrizes als geeignete Probenmaterialien für die PPRV-

Diagnostik anzusehen sind. Im Weiteren wurden drei Antigennachweismethoden hinsichtlich ihrer diagnostischen Verlässlichkeit und ihrer Eignung als Pen-Side Test evaluiert. Für diesen Zweck standen ein Antigen-ELISA (ID Screen® PPR Antigen Capture) und zwei *Lateral Flow Device* (LFD) Systeme (ID Rapid® PPR Antigen und PESTE-Test) zur Verfügung. Erwartungsgemäß zeigten die Nasen- und Maultupfer in den drei Antigen-Testsystemen bessere Ergebnisse als die im Vergleich analysierten Kottupfer. Die beiden IDvet-Testsysteme (Antigen-ELISA und LFD-ID Rapid) schnitten in unseren Studien mit einer Sensitivität von jeweils 75,0 % für alle untersuchten Proben am besten ab. Der LFD von Lillydale (PESTE-Test) erreichte hingegen nur eine Sensitivität von 53,3 %. Aus praktischer Sicht, lieferten die LFDs Ergebnisse bereits in weniger als 30 Minuten gegenüber etwa 2 Stunden für den Antigen-ELISA. Die LFDs sind zudem sehr einfach in der Handhabung und benötigen keine weitere Laborausstattung. Eine weitere Stärke einiger LFDs ist ihre Lagerfähigkeit bei Raumtemperatur, die den diagnostischen Einsatz im Feld (besonders in afrikanischen oder asiatischen Ländern) erheblich erleichtert.

In Abhängigkeit von den individuellen Anforderungen, entweder für die Routine-Diagnostik, umfangreiche Epidemiologiestudien oder für die POC-Diagnostik während eines Ausbruchgeschehens, stehen verschiedene Schnelltestsysteme für die Detektion von PPRV zur Verfügung, wovon einige in unseren Studien vergleichend getestet worden. In Bezug auf die Laborausstattung, Fähigkeiten der Anwender, Anforderungen an die diagnostische Leistungsfähigkeit (Sensitivitäten, Spezifitäten, Zeitansprüche), klinischer Verlauf der Infektion im Tier (zu erwartende geringlastige versus hochlastige Proben) und die individuellen Zielsetzungen haben die in unseren Studien validierten Tests ihren Nutzen für die Detektion von PPRV. Die hier dargelegten Ergebnisse sollen dabei als Entscheidungshilfe für den Endanwender bei der Auswahl des für ihn passenden diagnostischen Tests verstanden werden. Und das hier beschriebene Referenzproben-Panel wird dazu beitragen, zukünftige Testsysteme, wie z.B. molekulare POC-Testsysteme, zu optimieren und zu validieren.

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SUPPLEMENT

1 List of abbreviations

AGID	Agar gel immunodiffusion
B-ELISA	Blocking ELISA
C-ELISA	Competitive ELISA
CCPP	Contagious caprine pleuropneumonia
CDV	Canine distemper virus
CPE	Cytopathic effect
DIVA	Differentiation of infected from vaccinated animals
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FMDV	Foot-and-mouth disease virus
F gene	Fusion gene
F protein	Fusion protein
GTPV	Goatpox virus
HA	Haemagglutination
HN protein	Hemagglutinin-neuraminidase protein
IC-ELISA	Immunocapture ELISA

ICTV	International Committee on Taxonomy of Viruses
LFD	Lateral flow device
L protein	Large protein
mAb	Monoclonal antibody
Mccp	Mycoplasma capricolum subsp. Capripneumoniae
M protein	Matrix protein
N gene	Nucleocapsid gene
N protein	Nucleocapsid protein
OIE	Office International des Epizooties
PCR	Polymerase chain reaction
PDV	Phocine distemper virus
POC	Point-of-care
PPR	Peste des petits ruminants
P protein	Phosphoprotein
PPRV	Peste des petits ruminants virus
RP	Rinderpest
RPV	Rinderpest virus
RT-LAMP	Reverse transcription loop-mediated isothermal amplification
RT-PCR	Reverse transcription PCR
RT-qPCR	Real-time quantitative PCR

RT-RPA	Reverse transcription recombinase polymerase amplification
S-ELISA	Sandwich ELISA
SLAM	Signaling lymphocyte activation molecule
UAE	United Arab Emirates
VDS	Vero dog-SLAM
VNT	Virus neutralization test

2 List of figures

3 List of tables

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