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ORIGINAL ARTICLE

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

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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 analyse 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 (LFDs) 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, faecal samples were also analysed. 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-PESTE-TEST 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 faecal 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, peste des petits ruminants (PPR), rapid detection methods, *Small ruminant morbillivirus*, virus

1 | 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) (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 haemagglutinin–neuraminidase protein (HN), the large protein (L) and two non-structural proteins, C and V (Kumar et al., 2014).

In naïve populations, the disease induces a morbidity of up to 100% (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 4–6 days. Infected animals show a loss of appetite associated with high fever and oculo-nasal discharge, oral lesions, bronchopneumonia, cough, dyspnoea, gastroenteritis and diarrhoea. 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 (Baron et al., 2016; 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 neighbouring 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; 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; 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 (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 (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 (OIE, 2019; Abraham & Berhan, 2001; Abubakar et al., 2011; Couacy-Hymann, Bodjo, Koffi, Kouakou, & Danho, 2009; Diop, Sarr, & Libeau, 2005), 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 faecal swabs, as well as tissue samples (Bataille et al., 2019; ID.Vet, 2019bet, 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 (OIE, 2019; Couacy-Hymann et al., 2002). 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 to use the samples obtained after experimental infection to compare distinct matrices including EDTA blood, ocular, nasal, mouth and faecal swabs for their suitability for rapid testing.

2 | MATERIALS AND METHODS

2.1 | 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.

2.2 | PPRV isolates

The PPRV isolates for the animal trial 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 5,000 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, dyspnoea, diarrhoea and swelling of the inter-digital 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.

2.3 | 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 high-throughput sequencing on an Illumina platform. Raw data were analysed with the Genious software package v11.1.5 (Biomatters, Ltd., New Zealand).

2.4 | 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 4–6 months. Animals were infected with the UAE isolate ($10^{4.38}$ TCID50/ml) or the India isolate ($10^{5.75}$ TCID50/ml). Two millilitre of the inoculum was administered intranasally with a LMA™ MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex Medical, USA). Samples (EDTA blood, serum, ocular, nasal, mouth and faecal swabs) were collected at days −1, 3, 5, 7, 10, 12, 14, 17, 21 and 28 post-infection (dpi). In addition, faecal samples picked up from the stable floor for the individual animals were collected at irregular intervals. On −1 dpi, pooled faecal 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.

2.5 | 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 foetal calf serum using a 5 mm steel bead in the TissueLyser (Qiagen, Germany), and 100 µl homogenate was 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 TaqMan-probe (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.

2.6 | Serological and molecular biological investigation of EDTA blood, serum, ocular, nasal, mouth and faecal 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 was 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.

2.7 | 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

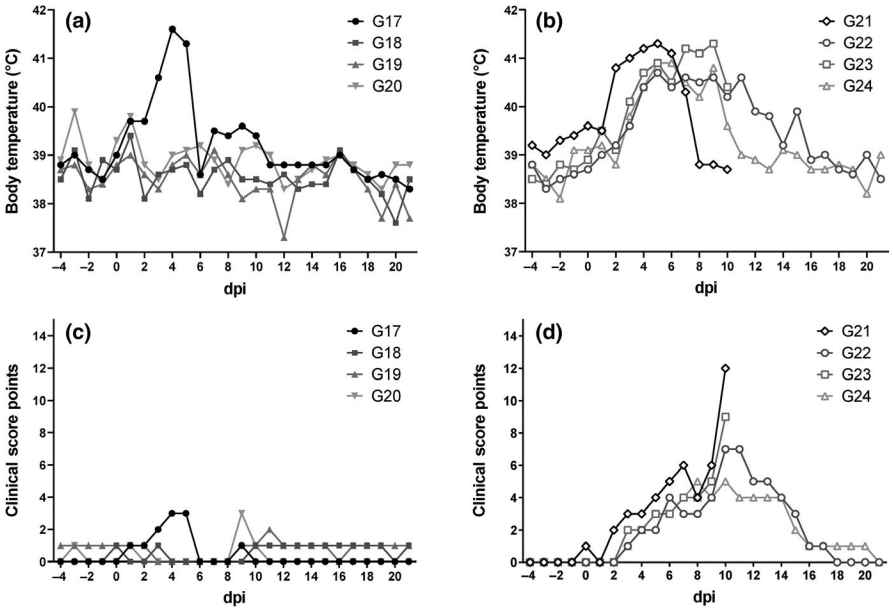

FIGURE 1 Clinical parameters of goats G17 to G24 during animal infection trial (−4 to 21 dpi): rectal body temperature for goat group 'India' (a) and goat group 'UAE' (b); average daily clinical score for goat group 'India' (c) and goat group 'UAE' (d)

manufacturer-specific buffers (subgroup 'IDvet/ Lillidale'). Mouth and faecal 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 and 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 faecal samples was prepared according to the instructions of the manufacturer IDvet, France. For this, 5 g of faeces was weighed into 1 ml of ID Rapid—Elution Buffer, vortexed and incubated for 5 min 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; and +++ = 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 with 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).

3 | RESULTS

3.1 | 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 1,324. 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%.

3.2 | 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–41.6°C) on 3–5 dpi. In two other goats, mild nasal discharges were noted.

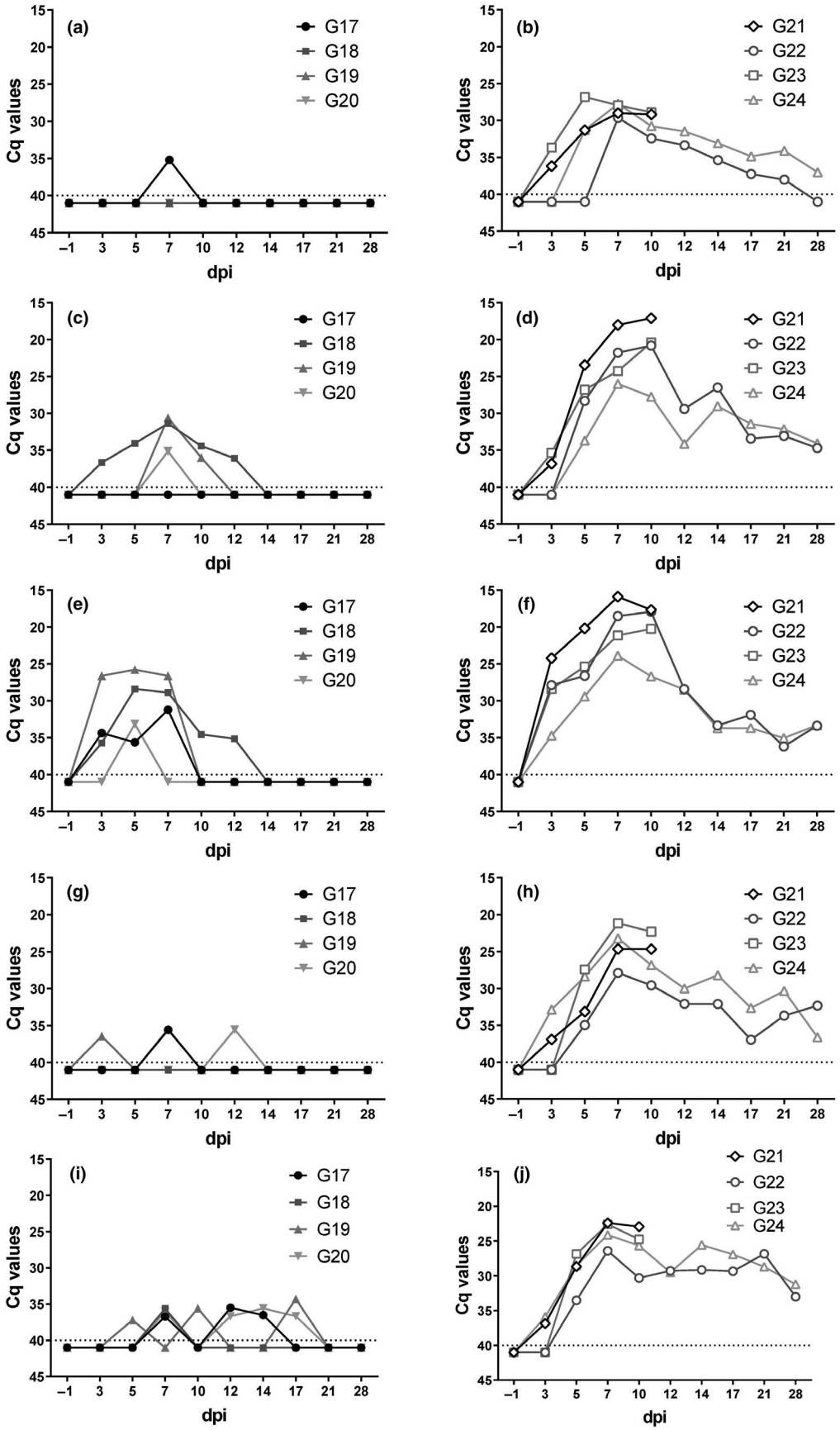

FIGURE 2 Loads of PPRV RNA in various sample matrices detected by RT-qPCR: left side (a, c, e, g, i) = 'India' group and right side (b, d, f, h, j) = 'UAE' group; EDTA blood = a + b; eye swabs = c + d; nasal swabs = e + f; mouth swabs = g + h; faecal swabs = i + j

Indeed, neither mucosal erosions nor any other severe clinical manifestations were observed (Figure 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 2–4 dpi and lasting for 6–8 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 3–7 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 diarrhoea. 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 (Figure 1).

3.3 | RNA loads in EDTA blood and swabs

Figure 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 3–7 dpi. However, generally viral genome loads were limited with lowest Cq values of 25–26. 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 30–31 on 7 dpi. EDTA blood and mouth swabs were inadequate exhibiting the lowest viral load similar to faecal 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 faecal 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 7–10 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 15–17 on 7 and 10 dpi. The highest viral loads in faecal swabs were detected on 7 dpi with Cq values of 22–26. Interestingly, in the faecal 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 (Figure 2).

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

3.4 | 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.

3.5 | 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 (Figure 3).

3.6 | 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–S5). Both LFDs and the antigen ELISA achieved 100% diagnostic specificity in all tested matrices and sample

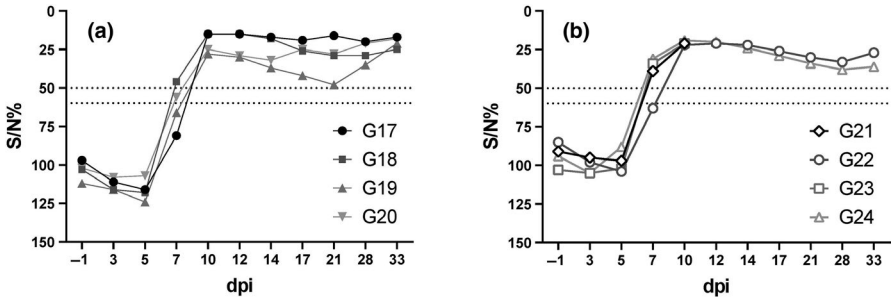

FIGURE 3 Serological results for goat group 'India' (a) and goat group 'UAE' (b) obtained with ID Screen® PPR Competition (IDvet, France)

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[a]			Buffer of the PESTE-Test[b]		
	Buffer solution	No. tested	LFD	ELISA	No. tested	LFD	ELISA
Positive samples							
Ocular swabs	Specific[c]	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[c]	9	7 (77.8%)	7 (77.8%)	8	6 (75.0%)	6 (75.0%)
	PBS	12	3 (25.0%)	3 (25.0%)	–	–	–
Faecal 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[d]	16	12 **(75.0%)**	12 **(75.0%)**	15	8 **(53.3%)**	
Negative samples							
Ocular swabs	Specific[c]	4	0 (100%)	0 (100%)	3	0 (100%)	0 (100%)
	PBS	3	0 (100%)	0 (100%)	–	–	–
Nasal swabs	Specific[c]	2	0 (100%)	0 (100%)	2	0 (100%)	0 (100%)
	PBS	2	0 (100%)	0 (100%)	–	–	–
Faecal samples	ID Rapid	13	0 (100%)	0 (100%)	–	–	–
Total		24	0 (100%)	0 (100%)	5	0 (100%)	0 (100%)

[a]swabs collected with the elution buffer of the IDvet kit or PBS were examined.

[b]swabs collected with the buffer solution of the Lillidale kit were examined.

[c]buffer solution used is specific according to the manufacturer's instructions of the LFD's.

[d]considers only those samples that have been taken in manufacturer-specific buffers and without faecal swabs to ensure comparability of the tests with each other.

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

4 | 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 with 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 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 4–7 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 3–7 dpi. Our study aimed to identify differences regarding optimal sampling between subclinically and acutely infected animals. For both groups, PPRV RNA was continuously detected only in nasal or ocular swabs. EDTA blood, mouth and faecal 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 faecal 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.

Faecal 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, faecal 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 faecal 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 (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 Table 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

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
Handling of the test in general			
Simplicity	++	+++	+[a]
Good runnability of the test strips	+++	+	n.a.
Duration (without preparation time)	30 min	30 min	2 hr
Supplied materials			
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
Storage of the kit			
Room temperature (18°C–30°C)	+++	–	–
Refrigerator temperature (2°C–8°C)	+	+++	+++
Results			
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

Note: +++, completely agree; ++, rather agree; +, is insufficient; –, strongly disagree; n.a., not applicable.
[a]For experienced users only.

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 well-suited 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 analyse 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 min 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–S4). The advantages of PESTE-Test are its simple handling, results are also available within 30 min 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 unfavourable 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.

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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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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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