

Tissue Distribution and Relative Quantitation of Experimental Infection with Peste Des Petits Ruminant Virus in Goats Using Real-Time PCR (TaqMan®)

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Abstract: Peste des Petits Ruminants (PPR) is an acute, highly contagious and economically important viral disease of small ruminants, especially goats and sheep. Given the contagious nature of the disease, speed of diagnosis is essential in order to quarantine infected animals and initiate appropriate prophylactic measures. Here researchers reported the distribution of PPRV RNA in various tissues of goats experimentally infected subcutaneously with a vaccine virus (Nigeria 75/1) strain in relation to the time post inoculation (pi) by real-time PCR (rt RT-PCR) based on TaqMan® and was quantified as a fold change ($2^{-\Delta\Delta C_t}$ values) compared with that of blood at 3 dpi. PPRV levels at 3, 5 and 28 dpi were higher than those at the other time points, especially at 5 dpi. The PPRV level in thymus, tonsil, hilar lymph node and turbinate bone was higher than that of other tissues. The result suggested that the distribution of PPRV in goats were similar to that of viral antigens and rt RT-PCR was useful for the detection of PPRV in field samples. The higher levels of RNA detected at 28 dpi may suggest some level of persistent infection. Furthermore, it was shown that blood sample was the most valuable diagnostic material for use in an epidemic situation.

Key words: Peste des petits ruminant virus, tissue distribution, goat, real-time PCR, Tymph, China

INTRODUCTION

Peste des Petits Ruminants (PPR) is an acute, highly contagious and economically important viral disease of small ruminants, especially goats and sheep. It is considered as a rapidly spreading, transboundary disease and has a high economic impact on trade. Morbidity and mortality are variable but can reach 90-100% when the disease occurs in naive populations of small ruminants (Diallo *et al.*, 2007). PPR was first reported in the Ivory Coast in the 1940s and it can now be detected in a broad belt of sub-Saharan Africa, the Arab Peninsula, the Middle East, Asia and Europe (Dhar *et al.*, 2002; Singh *et al.*, 2009; Banyard *et al.*, 2010). PPR Virus (PPRV) belongs to the genus *Morbillivirus*, family Paramyxoviridae along with Rinderpest Virus (RPV),

Canine Distemper Virus (CDV), Measles Virus (MV) and Phocine Distemper Virus (PDV). PPRV has a single-stranded, negative-sense RNA genome that encodes eight proteins in the order: 3'-N-P/C/V-M-F-H-L-5' (Bailey *et al.*, 2005). The molecular epidemiology of PPRV based on the *F* gene sequence has defined the presence of four different lineages of the virus worldwide: PPRV isolated from Senegal, Sudan and Nigeria were grouped as lineage I, those from the Ivory Coast and New Guinea as lineage II, those from Oman, Sudan and India (TN/92) as lineage III and the other strains circulating across Asia as lineage IV (Dhar *et al.*, 2002; Shaila *et al.*, 1996; Wang *et al.*, 2009). PPRV infection has emerged in goats and sheep and was officially reported in the Ngari region of Southwestern Tibet, People's Republic of China in July 2007. The PPRV strain from Tibet is classified in lineage IV

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and it is closely related to viruses circulating currently in neighboring countries of Southern Asia (Wang *et al.*, 2009).

Given the contagious nature of the disease, speed of diagnosis is essential in order to quarantine infected animals and initiate appropriate prophylactic measures. PPRV, like other morbillivirus infections, requires close contact between infected and susceptible animals to spread (Lefevre and Diallo, 1990). The discharges from eyes, nose and mouth and fecal material are the sources of virus infection (Singh *et al.*, 2004a). The infectious materials can also temporarily contaminate water and feed troughs, bedding materials, etc. turning them into additional sources of infection (Singh *et al.*, 2004b).

In the last two decades, the structure and biology of PPRV has become well known which has permitted the development of specific and sensitive tests for rapid diagnosis. Immunohistochemical (IHC) test and Enzyme-Linked Immunosorbent Assays (ELISAs) using monoclonal antibodies have been used for serological diagnosis in the competitive format and for specific antigen detection by immunocapture (Singh *et al.*, 2004a; Anderson and McKay, 1994; Libeau *et al.*, 1994; Zhang *et al.*, 2012; Forsyth and Barrett, 1995). Several Reverse Transcription-PCR (RT-PCR) Methods based on different genes have been developed for the rapid and specific detection of PPRV (Couacy-Hymann *et al.*, 2002; Balamurugan *et al.*, 2006; Bao *et al.*, 2008). In the past few years, real-time RT-PCR and Loop-mediated isothermal Amplification (LAMP) have been developed to detect PPRV with higher specificity and selectivity (Balamurugan *et al.*, 2010; Kwiatek *et al.*, 2010; Li *et al.*, 2010; Couacy-Hymann *et al.*, 1995).

Among the genes of morbilliviruses, the Nucleoprotein (N) gene is the most abundant gene transcribed in virus-infected cells; there is a steep gradient from the 3'-5' end of the genome. Given that it is also a well-conserved gene within the Paramyxoviridae family, the N gene is probably one of the best targets for the development of a highly sensitive real-time RT-PCR. The N protein has proved to be a good candidate for developing differential diagnosis tests for RPV and PPRV.

In the present study, the aim was to investigate the distribution of viral RNA to different organs in relation to the day post infection (dpi) and to assess the diagnostic values of a TaqMan rt RT-PCR techniques and different field samples in the diagnosis of PPR infections. In order to detect the distribution, goats were euthanized and necropsied at predetermined days after subcutaneous inoculation with the PPR vaccine virus (Nigeria 75/1) strain and the PPRV RNA was detected by a real-time RT-PCR (rt RT-PCR) based on the N gene using the TaqMan® technology.

MATERIALS AND METHODS

Viruses and cells: The attenuated PPR vaccine virus (Nigeria 75/1) (Worrall *et al.*, 2000; Livak and Schmittgen, 2001) was used in the study. The virus was obtained from the virus bank of the China Institute of Veterinary Drugs Control. Vero cells were grown in DMEM medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine calf serum and a confluent Vero cell monolayer was used for propagation of the virus.

Animals and experimental infection: Twenty one healthy goats aged between 6 and 8 months and weighing between 13 and 15 kg were obtained from an experimental livestock provider. They tested negative for the presence of antibodies against PPRV by the virus-neutralization test. Researchers divided the 21 goats randomly into two groups: one experimental group of 18 goats and one control group of 3 goats. Each animal in the experimental group was infected subcutaneously with 1 mL of the challenge viral suspension at a concentration of 10^5 TCID₅₀ mL⁻¹. Each group of animals was kept separately in a specific cage in the laboratory-animal house. Each cage was identified with a specific number and had a separate attendant who was assigned blindly, to feed and water the animals. The animals were examined daily and the rectal temperature was recorded daily. Three goats in the experimental group were euthanized at 1, 3, 5, 7, 14 and 28 dpi. All animal experiments were performed according to the protocols approved by the institutional committee for the use and care of animals.

With the exception of blood samples which were stored in 3.8% citrate sodium solution, 44 fresh samples of various tissues were rinsed in Phosphate Buffered Saline (PBS; pH 7.2), placed in RNALater (Qiagen, Germany) and stored at -80°C until use. The samples included abomasum, abdominal muscles, adrenal gland, blood, cecum, cerebrum, cerebellum, colon, dorsal muscles, duodenum, esophagus, feces, gallbladder, gluteus, gonad, gum, heart, hilar lymph nodes (l.n.), ileum, inguinal l.n., injected muscle, jejunum, kidney, larynx, lip, liver, lung, mandibular l.n., mesenteric l.n., nasal mucosa, omasum, pancreas, rectum, reticulum, rumen, soft palate, spinal cord, spleen, superficial cervical l.n., thymus, tongue, tonsil, trachea, turbinate bone and urinary bladder.

Design of primers and probe: Using multiple sequence alignments, the oligonucleotide primers and probe used for real-time PCR amplification of PPRV were designed with Primer Express 2.0 Software using the N gene sequence of the Nigeria 75/1 PPR vaccine strain as

Table 1: Fold difference in level of N in different tissues of goats infected experimentally with Nigeria 75/1, by $\Delta\Delta Ct$ Method

Tissues\Times	1 day	3 days	5 days	7 days	14 days	28 days	Tissues\Times	1 day	3 days	5 days	7 days	14 days	28 days
Abdominal muscles	0.000	0.000	0.000	0.000	0.000	0.000	Lip	0.318	0.069	0.032	0.017	0.021	0.031
Abomasum	0.009	0.017	0.021	0.100	0.011	0.010	Liver	2.140	23.710	5.910	0.000	0.000	1.970
Adrenal gland	0.789	0.943	1.320	0.459	0.089	0.191	Lung	2.560	1.870	0.904	0.218	0.057	1.170
Blood	0.900	1.000	1.400	0.250	0.240	0.000	Mandibular l.n.	0.050	3.970	43.330	0.330	0.000	1.250
Cecum	0.000	7.150	2.190	1.110	0.000	0.371	Mesenteric l.n.	0.000	1.329	25.180	0.406	0.309	0.670
Cerebellum	0.385	3.950	18.700	2.570	1.920	1.790	Nasal mucosa	0.192	0.480	25.600	0.536	0.247	19.000
Cerebrum	0.000	0.000	0.750	0.000	0.000	0.000	Neck muscles	0.058	0.025	0.014	0.000	0.000	0.000
Colon	0.000	0.000	2.570	0.000	0.000	0.670	Omasum	0.109	0.163	0.040	0.002	0.002	0.005
Dorsal muscles	0.000	0.000	0.000	0.000	0.000	0.000	Pancreas	0.000	0.000	0.000	0.000	0.000	0.000
Duodenum	0.020	0.076	1.270	0.022	0.012	0.011	Rectum	0.870	0.000	0.000	0.000	0.000	0.000
Esophagus	1.580	2.860	11.500	0.804	0.252	7.830	Reticulum	0.032	0.051	0.054	0.066	0.012	0.098
Feces	0.000	0.000	0.000	0.000	3.000	1.830	Rumen	0.005	0.016	0.029	0.020	0.007	0.048
Gallbladder	0.000	2.200	0.893	3.520	3.240	0.498	Soft palate	0.014	6.920	0.053	0.086	0.005	0.002
Gluteus	0.000	0.000	0.000	0.000	0.000	0.000	Spinal cord	0.000	3.140	5.370	4.358	0.000	1.348
Gonad	1.335	9.875	14.075	0.420	0.000	1.805	Spleen	0.335	1.200	2.650	0.394	0.170	0.204
Gum	0.047	0.082	0.076	0.013	0.001	0.009	Submandibular gland	0.024	0.620	1.230	0.163	0.059	0.080
Heart	1.910	7.75	30.800	0.606	0.248	0.391	Superficial cervical l.n.	0.934	7.850	7.580	0.388	0.572	0.796
Hilar l.n.	1.020	86.00	207.000	18.000	5.420	29.00	Thymus	7.270	247.000	83.300	5.230	0.626	1.310
Ileum	0.020	0.336	0.378	0.010	0.020	0.027	Tongue	0.056	5.176	6.040	0.000	0.000	1.866
Inguinal l.n.	0.000	1.450	4.110	0.131	0.557	0.959	Tonsil	2.720	2.120	225.000	0.438	0.127	1.040
Jejunum	0.020	0.106	0.329	0.058	0.009	0.014	Trachea	2.630	0.796	0.644	0.106	0.048	0.563
Kidney	0.359	0.826	1.280	0.137	0.066	1.580	Turbinate bone	49.200	124.000	43.300	39.500	18.800	41.300
Larynx	0.125	0.123	0.317	0.050	0.053	0.064	Urinary bladder	0.703	7.350	5.400	1.850	0.290	0.532

template (Accession No. X74443). β -actin was used as an endogenous control the primers and probe were developed using caprine β -actin sequences available in GenBank (Accession No. AF481159). Primers and probes were synthesized and purified by TaKaRa (TaKaRa Dalian Biotechnology Co., Ltd. Dalian, China). The position and sequence of the primers and probes are reported in Table 1. The reporter dye (FAM) was measured against the internal reference dye (ROX) to normalize the signals for differences in non-PCR related fluorescence that occurred from well to well.

Preparation of RNA: Total RNA was extracted from samples (25 mg) with the RNeasy kit (Qiagen GmbH, Hilden, Germany) and digested with RNase-free DNase I (Promega) to eliminate the genomic contamination. PCR of treated RNAs was carried out by special primers and samples with negative results were used in the following procedure. To rule out carryover contamination, negative controls included the RNA extracted from biological samples from healthy goats and the No-Template Control (NTC) used nuclease-free water as template. The quality and concentration of RNA were determined using the OD260/280 ratio measured by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and stored at -80°C prior to use.

Reverse transcription: Reverse Transcription (RT) was carried out using a PrimeScript™ RT Reagent kit (TaKaRa, Japan). About 1 μ g of template RNA was reverse transcribed in a reaction volume of 20 μ L

containing PrimeScript™ buffer 2 μ L (5 \times), PrimeScript™ RT Enzyme Mix I 0.5 μ L, Oligo dT 25pM and random 6-mers 200 pM. Synthesis of cDNA was carried out at 37°C for 15 min and 85°C for 5 sec.

Quantitative real-time PCR: Real-time PCR was performed on Mx3000P™ real-time PCR machine (Agilent Stratagene, California, USA) using the Premix Ex Taq™ kit (TaKaRa, Japan). The PCR was carried out using optical 96 well reaction plates covered with plate sealers. Each tube of TaqMan reaction mix contained 2 μ L of RT cDNA, 12.5 μ L of 2 \times Premix Ex Taq™, 1.25 μ L of 3 μ M Taqman probe, 0.6 μ L of 10 μ M forward, 0.6 μ L of 10 μ M reverse primers and 0.5 μ L of 50 \times ROX Reference Dye II. Each reaction was prepared in triplicate. The program was run using the following universal cycling conditions: one cycle at 95°C for 30 sec followed by 45 cycles at 95°C for 5 sec and 60°C for 20 sec. The N gene in blood samples at 3 dpi was used as a calibrator. A No-Template Control (NTC) was prepared using only water. The calibrator and NTC were tested along with the unknown samples in each run.

Generation of standard RNA: In order to validate the TaqMan assay, a series of 5 fold dilutions of the total RNA from blood samples was prepared to generate a standard curve. A 5 fold dilution series of 20 and 4 ng, 800, 160, 32 and 6.4 pg total RNA from blood samples in 2 μ L medium was used as the template in one tube. The serial dilutions were made using EASY dilution (TaKaRa, Japan). The reaction volume was 2 μ L of each

dilution in 25 μ L of final volume for PCR. Three replicates of each dilution were amplified following the conditions described in the study.

Analysis of real-time PCR data: The PCR data were analyzed using the Comparative Ct Method ($\Delta\Delta C_t$) (Choi *et al.*, 2005). The C_t was recorded for the expression of each gene assayed in the real-time PCR system using the TaqMan probe. All the C_t values were the means of triplicate samples tested. Caprine β -actin was chosen as the reference gene for the internal control. The difference between the C_t values of the target gene and the internal control gene ($\Delta C_t = C_t \text{ target} - C_t \text{ internal control}$) were calculated to normalize the differences in the amount of total cDNA added to each reaction and the efficiency of the RT-PCR. The blood sample was used as the calibrator for each comparison to be made. Differences between the ΔC_t of each sample and calibrator: $\Delta\Delta C_t = ((C_t \text{ target} - C_t \text{ internal control}) \text{ sample} - (C_t \text{ target} - C_t \text{ internal control}) \text{ blood})$ were calculated. The change in the gene expression was calculated as $2^{-\Delta\Delta C_t}$ and the value represented an n fold difference relative to the blood sample at 3 dpi.

RESULTS

Standard curve for real-time PCR: Serial 5 fold dilutions of total RNA were reversed transcribed. The cDNAs were used to determine the detection rate and the linearity of

the assay (Fig. 1 and 2). The standard curve generated covered a linear range of six orders of magnitude and showed linearity over the entire quantitation range (Fig. 1a and b, N, slope = -3.333; Fig. 1c and d, β -actin, slope = -3.383), providing an accurate measurement over a range of initial target concentrations. The coefficient of linear regression (R^2) was equal to 0.999 and 0.996 and the PCR efficiency was 99.5 and 99.1%, respectively.

The cDNA fragments with the expected size for *N* gene and β -actin were amplified and no primer dimers were visible. To confirm their specificity, the real-time PCR products were sequenced and showed 100% identity with the *N* gene of PPRV. No detectable fluorescence signal was obtained in NTC tubes which confirmed that the assay was specific for the detection of the *N* gene.

Tissue distribution and relative quantitation of PPRV in various tissues of goats: With the exception of edematous lymph nodes, the clinical and necropsy findings showed that all animals were clinically normal. The cDNA fragments with the expected size for viral RNA and β -actin were amplified and no primer dimers were visible. This analysis showed that PPRV RNA was detected, albeit at varying levels, in the 45 tissue samples. The PPRV RNA of blood at 3 dpi was set equal to 1 and all other tissues were quantified as a fold change ($2^{-\Delta\Delta C_t}$ values) compared with PPRV RNA in blood at 3 dpi (Table 1). A rt RT-PCR for the detection of PPRV in tissue samples was developed. To confirm the specificity, the PCR products

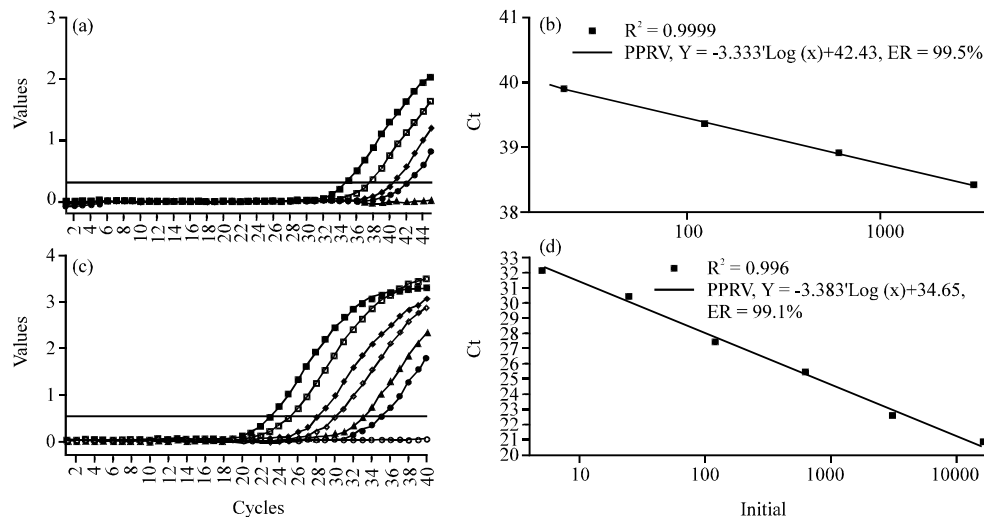


Fig. 1: Amplification plots and standard curve of the real-time PCR assay for gene *N* of a, b) PPRV and c, d) β -actin. The 5 fold dilutions of standard RNA prior to amplification were used as indicated on the y-axis and the corresponding Cycle threshold (C_t) values are presented on the y-axis. Each dot represents the result of triplicate amplification of each dilution. The coefficient of determination (R^2) and the slope value (s) of the regression curve were calculated and are indicated

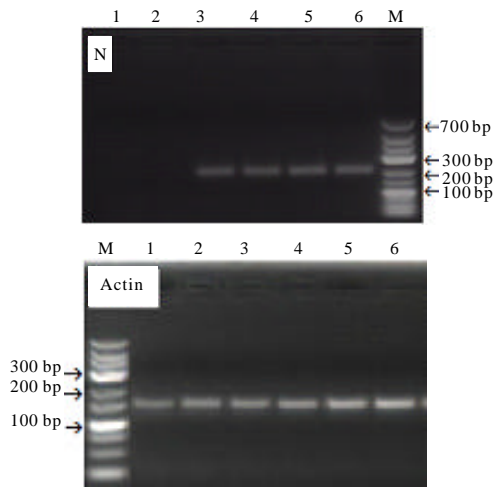


Fig. 2: Sensitivity of real-time RT-PCR based on the *N* gene of PPRV and β -actin. Lane M, GeneRuler™ Lower Range DNA Ladder (Fermentas, GmbH, St. Leon-Rot., Germany); Lanes 1-6, 5 fold dilutions of blood sample at 3 dpi

were sequenced and they showed 100% identity with the *N* gene sequence of the Nigeria 75/1 PPR vaccine strain. The results showed that PPRV RNA levels at 3, 5 and 28 dpi were higher than those of the other time points, especially at 5 dpi. The higher levels of RNA detected at 28 dpi may suggest some level of persistent infection. It remains to be studied further. PPRV RNA was not detected in the pancreas, gluteus, dorsal muscle or abdominal muscle at different time points during the experiment. PPRV nucleic acid was detected at high levels in cecum, cerebellum, esophagus, gonad, heart, liver, lymph nodes (hilar l.n., mandibular l.n., mesenteric l.n. and superficial cervical l.n.), nasal mucosa, soft palate, spinal cord, thymus, tongue, tonsil and turbinate bone. The profiles of PPRV in adrenal gland, abomasum, colon, duodenum, gum, ileum, inguinal l.n., injected muscle, jejunum, kidney, larynx, lip, omasum, rectum, reticulum, rumen and spleen were similar except that in lung and trachea: PPRV in these tissues was detected at 5 fold lower levels than that in blood at 3 dpi. The RNA level of virus in urine was not investigated. The level in different tissues was increased slightly at 28 dpi after the lowest level was detected at 14 dpi. PPRV was not detected in any tissues of the control group.

DISCUSSION

PPR is an acute and highly contagious viral disease of small ruminants which is characterized by high fever, ocular and nasal discharge, pneumonia, necrosis and

ulceration of the mucous membranes and inflammation of the gastrointestinal tract leading to severe diarrhea. PPR infection has been recognized in many Asian countries (Banyard *et al.*, 2010). Almost all viruses isolated recently from Southwest Asia and the Middle East belong to PPRV lineage 4 (Wang *et al.*, 2009).

Given the recent outbreaks of PPR in new areas of the world, it is essential to develop an efficient protocol for surveillance of PPRV in developing countries. Currently, the diagnosis of PPRV infection from clinical samples is based on the detection of specific immunoglobulin, virus isolation or genomic detection (Bao *et al.*, 2008; Batten *et al.*, 2011). The detection and quantitation of target genes with high sensitivity, offers great advantages over the classical nucleic acid methods. In recent years, several nucleic acid amplification techniques have been developed for the rapid, specific and sensitive detection and identification of PPRV such as RT-PCR, nested PCR, rt RT-PCR and RT-LAMP (Balamurugan *et al.*, 2006; Bao *et al.*, 2008; Balamurugan *et al.*, 2010; Kwiatak *et al.*, 2010; Li *et al.*, 2010; Couacy-Hymann *et al.*, 1995; Brown *et al.*, 1991). Bao reported the sensitivity of a rt RT-PCR based on nucleocapsid (*N*) gene which allowed quantification and detection of a viral RNA concentration of 8.1 RNA copies per reaction mixture (Balamurugan *et al.*, 2010). Li reported that the detection limit of the RT-LAMP assay for PPRV developed was 10-fold higher than that for RT-PCR and similar to that of the rt RT-PCR assay (Couacy-Hymann *et al.*, 1995). In this study, a two-step rt RT-PCR assay was developed and evaluated for the detection of PPRV in field samples. The result showed that the two-step rt RT-PCR had a high diagnostic efficacy for the detection of PPRV nucleic acid in the experimental samples as early as 1st dpi.

It is emphasized that the detection of PPRV nucleic acid in clinical samples such as blood, nasal swab and conjunctival swab and in necropsy materials such as lymph node, spleen and lung is of great value in the diagnosis of PPRV infection by RT-PCR. Earlier studies showed that lymphoid tissues were found to be the main target for virus replication (Eligulashvili *et al.*, 1999; Kumar *et al.*, 2004; Saliki *et al.*, 1994; Kul *et al.*, 2007). The typical tissue distribution has been shown by immunolabeling of the viral antigen. Antigen was observed in the kidney, brain, rumen, abomasum, heart and myocytes of the tongue, besides its more typical locations (FAO, 1999).

In this study, different materials at different stages of infection, such as blood, swab samples (nasal, conjunctival and oral) and tissue samples were used to detect the distribution and quantify the level of PPRV RNA in experimentally infected goats. The results showed

that PPRV levels at 3, 5 and 28 dpi were higher than those at other times, especially at 5 dpi. The tissue distribution of viral nucleic acid was similar to that of viral antigens (Saliki *et al.*, 1994; Galbraith *et al.*, 2002; Toplu, 2004; FAOUN, 1999; Wohlsein *et al.*, 1993). Viral nucleic acid was detected at high levels in the tongue, soft palate, nasal mucosa, turbinate bone, esophagus, cecum, lymph nodes (superficial cervical l.n., mandibular l.n., hilar l.n. and mesenteric l.n.), heart, liver, tonsil, thymus, gonad and spinal cord. Forsyth and Barrett reported that viral nucleic acid was detected in samples of blood, conjunctival swab, lymph node and spleen by RT-PCR (Couacy-Hymann *et al.*, 2002). However, they did not detect viral nucleic acid in samples of lung, oral swab and nasal swab so, their results were inconsistent with those of this study. In addition, this study revealed a novel distribution of the viral nucleic acid in abomasum, adrenal gland, colon, duodenum, gums, ileum, injected muscle, jejunum, kidney, larynx, omasum, rectum, reticulum, rumen and spleen. This may have been due to the differing sensitivity of different methods. The findings from the study suggest that blood and lymph nodes are of great value in the diagnosis of PPRV infection by rt RT-PCR. Earlier research showed that the lung was an important target organ for most morbillivirus infections (Cosby *et al.*, 2002). PPRV antigen was detected particularly in alveolar macrophages and in syncytial and bronchial epithelial cells of the lungs in the oral mucosa and in reticular cells and sinusoidal macrophages of lymphoid tissues (Eligulashvili *et al.*, 1999; Saliki *et al.*, 1994; Wohlsein *et al.*, 1993). This study revealed viral nucleic acid in the lung from 1-14 dpi with the amount decreasing over time. At 1 and 3 dpi, viral nucleic acid was detected in the lung at 2.56 and 1.87 fold higher levels than in blood at 3 dpi at other times the amount was less than in blood at 3 dpi. This result suggested further that blood is the most valuable diagnostic material for use in the epidemic situation.

All morbilliviruses can infect the Central Nervous System but some morbilliviruses are eliminated easily by the developing host immunity and temporary central nervous system infection occurs (Toplu, 2004; Reuter and Schneider-Schaulies, 2010; Stimmer *et al.*, 2010; Park *et al.*, 2011; Wolfson *et al.*, 1991). However, the neurovirulence of PPRV still remain obscure because there are insufficient data available. Galbraith reported that PPRV (Nigeria 75/1) and RPV (Saudi/81) showed neurovirulence when the viruses were inoculated experimentally into the brains of mice (Toplu, 2004). In the study, researchers detected viral nucleic acid in the cerebellum during the experiment. This may indicate the potential of the virus to pass into the cerebrospinal fluid.

The molecular basis of persistent infection was characterized by alterations of viral gene expression on the transcriptional and translational levels. Earlier reports demonstrated that the persistent infection of murine neuroblastoma cells with MV resulted in a spectrum of changes in the expression of cellular genes and elevated level of MHC class I glycoproteins on plasma membrane (Wolfson *et al.*, 1991; Gopas *et al.*, 1992). Subacute Sclerosing Panencephalitis (SSPE) and Measles Inclusion Body Encephalitis (MIBE) were caused by a persistent Measles Virus (MV) infection (Reuter and Schneider-Schaulies, 2010; Woelk *et al.*, 2002; Baczko *et al.*, 1993; Abdullah *et al.*, 2009; Schubert *et al.*, 2006). MV can lead to a persistent infection in BALB/c mice for about 21 days (Schubert *et al.*, 2006). In present study, the higher levels of PPRV RNA detected at 28 dpi may suggest some level of persistent infection. Further research is needed to confirm this supposition.

CONCLUSION

This study is thought to be a detailed report of the distribution of PPRV nucleic acid in cases of experimental infection. The findings in the respiratory, digestive and immune systems were similar to those of studies investigating the distribution of viral antigen in PPRV infection. The result showed that PPRV might lead to a persistent infection in goat for about 28 days. This study suggested that two-step rt RT-PCR had a high diagnostic efficacy for the detection of PPRV nucleic acid in tissue samples and blood sample was the most valuable diagnostic material for use in an epidemic situation.

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