# The extraction of Peste Des Petits ruminants Virus RNA from paraffin-embedded tissues using a modified extraction method

**Research Article**

**Murat Şevik**

[1]Department of Virology, Veterinary Faculty, Necmettin Erbakan University, Konya, Turkey

ORCİD-
0000-0002-9604-3341

***Correspondence***
**Murat ŞEVİK**
murat.sevik@erbakan.edu.tr

## ABSTRACT

Peste des petits ruminants (PPR) which is caused by small ruminant morbillivirus (PPRV) has an important economic impact on small ruminant farming. Molecular assays are commonly used in the diagnosis of the disease. Extraction of RNA from formalin-fixed paraffin-embedded (FFPE) tissues is challenging because of the RNA is often degraded by formalin fixation process. Although commercial kits have been developed for extraction of nucleic acids from FFPE tissues, they are expensive than other extraction kits. In this study, a modified extraction method was evaluated for detection of PPRV from FFPE tissues. A total of 20 FFPE tissue samples including 15 PPRV positive and 5 PPRV negative FFPE tissue samples were used. Two years ago, these selected FFPE tissue samples were analysed by nucleoprotein gene based real time RT-PCR method before they were fixed with formalin and embedded in paraffin. FFPE tissue samples were extracted using modified extraction method and were tested by fusion (F) gene based one step RT-PCR. PPRV specific RNA was detected in 12 FFPE tissue samples whereas 3 positive samples were found negative by one-step RT-PCR. Furthermore, 5 negative FFPE tissue samples were also found negative. Three false negative results were from samples with high real-time RT-PCR cycle threshold. Therefore, false negative results could be related with lower viral loads which might be lower than detection limit of the one-step RT-PCR. The results of the study show that modified extraction method could be used for RNA extraction from FFPE tissues which had been stored for 2 years.

**Keywords:** Formalin-fixed tissues, peste des petits ruminants, RNA, RT-PCR

## INTRODUCTION

Peste des petits ruminants (PPR) is an economically important transboundary viral disease of sheep and goats characterized by high fever (40°- 41.5°C), diarrhoea, necrotic stomatitis, muco-purulent nasal discharge, enteritis and abortion (Couacy-Hymann, 2015; OIE, 2022; Şevik and Sait, 2015). Primary hosts of the disease are sheep and goats. Although cattle, pigs and camels can also be infected, clinical signs are not seen in these animals (Abraham et al., 2005; Fakri et al., 2019). The disease has also been reported in wild ruminant species including ibexes, gazelles, wild goats (*Capra aegagrus*) and sheep (*Ovis orientalis*) (Abubakar et al., 2011; Dou et al., 2020; Li et al., 2017; Mahapatra et al., 2015).

PPR causes high mortality rates in naïve goat and sheep populations (Couacy- Dou et al., 2020; Hymann et al., 2007; Şevik and Sait, 2015). In endemic areas, mortality rates may be 20% or less, but it may reach 100% naive population (Mapaco et al., 2019). The disease is spread through direct contact with infected animals or contact with infected fomites (Parida et al., 2019).

***Article info***
*Submission*: 23-02-2022
*Accepted*: 12-07-2022
*Online First:* 06-08-2022
*Publication:* 31-08-2022

***e-ISSN:*** 2548-1150
***doi prefix:*** 10.31797/vetbio
- http://dergipark.org.tr/vetbio



***How to cite this article***
**Şevik M. (2022).** The extraction of Peste Des Petits ruminants Virus RNA from paraffin-embedded tissues using a modified extraction method. *Journal of Advances in VetBio Science and Techniques*, *7*(2), 202-209. https://doi.org/10.31797/vetbio.1078235

The disease has been reported in southern Asia, Africa, the Arabian Peninsula and the Middle East (Banyard et al., 2014; Kerur et al., 2008; Li et al., 2017; OIE, 2022). Turkey first reported PPR in 1999 (OIE, 1999). Due to its economic impact, PPR has been declared as a notifiable disease by the World Organization for Animal Health (OIE) (OIE, 2022). The Food and Agriculture Organization and OIE aim to eradicate PPR until 2030 (Dou et al., 2020).

Peste des petits ruminants virus (PPRV), renamed as small ruminant morbillivirus, is the causative agent of the disease classified in the genus *Morbillivirus* within the family *Paramyxoviridae*, and closely related with rinderpest, measles and canine distemper viruses (Gibbs et al., 1979; ICTV, 2022). Four genetically distinct lineages of PPRV have been identified based on molecular characterization of the nucleoprotein (N) and fusion protein (F) of PPRV (Bailey et al., 2005; Kerur et al., 2008).

Virus isolation, immunocapture enzyme-linked immunosorbent assay, agar gel immunodiffusion, immunohistochemistry and molecular detection methods are routinely used for confirmation of the disease (OIE, 2022). Molecular detection methods are highly specific and sensitive. However, these methods require high-quality RNA from the extracted samples. Mostly, fresh tissues and swab specimens are used for the detection of PPRV nucleic acid (Şevik, 2014). However, it may not be possible to find fresh tissue samples in some cases. Therefore, in this study, a commercial kit which developed for total nucleic acid isolation from fresh tissues was modified, and it was evaluated for detection of PPRV from formalin-fixed paraffin-embedded (FFPE) tissues.

## MATERIAL and METHOD

### *Samples*

This study was conducted at Konya Veterinary Control Institute in 2017. A total of twenty FFPE lung tissue samples that were fixed with formalin and embedded in paraffin two years ago were selected. Selected tissue samples were analysed by N gene based one step real time RT-PCR method before they were fixed with formalin and embedded in paraffin. One step real time RT-PCR method was performed using primers and probe described by Batten et al. (2011) with the One-Step RT-PCR Kit (Qiagen, Hilden, Germany). Reaction mixture was prepared containing 0.4 µM of each primer and 5 µl of the extracted RNA, in a final volume of 25 µl. Amplification protocol was performed according to Batten et al. (2011) using a real time PCR machine (Roche Applied Science, Indiana, USA).

Of the 20 FFPE tissue samples, 15 samples were detected as positive whereas 5 samples were detected as negative by one step real time RT-PCR method. Samples that were positive by real-time RT-PCR had cycle threshold (Ct) values ranged from 24 to 39 (Figure 1). A FFPE lung tissue sample that was border disease virus positive was used as negative control for the analyses.

### *RNA Extraction*

Extraction of RNA was carried out from the FFPE samples using a commercial kit which developed for total nucleic acid isolation from fresh tissues (Roche Applied Science, Indiana, USA). However, extraction kit procedure was modified to obtain enough quality RNA from the FFPE tissue samples.

First, 5 µm paraffin tissue sections were collected from FFPE tissue samples, and were put into the eppendorf tubes. Then, they were deparaffinized by addition of xylene (1200 µl) and were incubated for 15 min at 65°C, followed by centrifugation for 5 min at 14000 × rpm. Deparaffinization steps were repeated three times. Then, samples were centrifuged for 5 min at 14000 × rpm with adding 80%, 90% and 100% ethanol, respectively. After samples washed with 100% ethanol, ethanol was

aspirated for 30 min at 37°C. Proteinase K (1:20) was added to tissue pellets which were into eppendorf tubes, and they were incubated at 56°C overnight. To inactivate proteinase K, samples were incubated for 15 min at 100°C before the RNA extraction procedure. After proteinase K inactivation, RNA extraction was performed according to the manufacturer's instructions. RNA extracts were stored at -20°C until analyses.

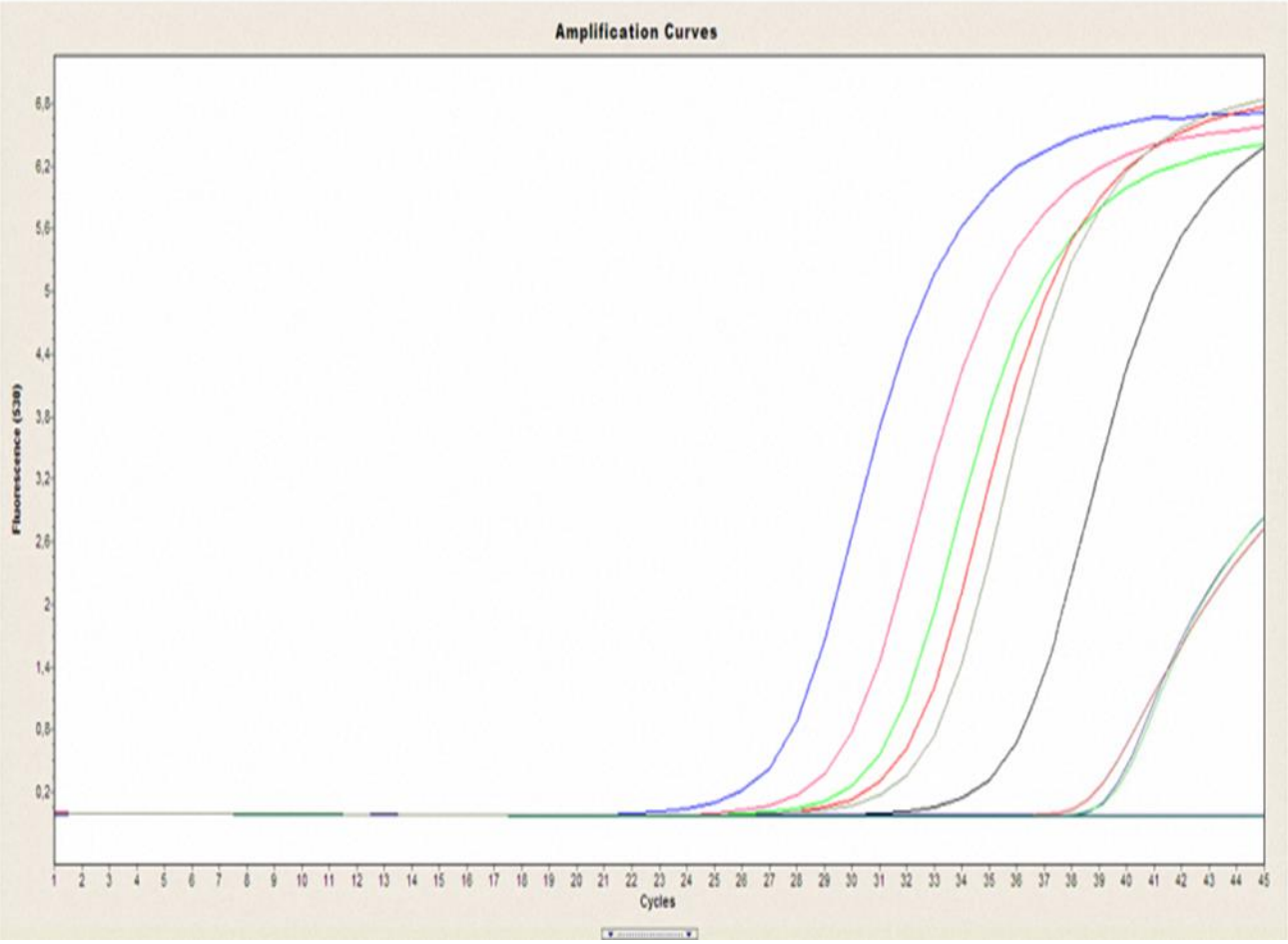


**Figure 1.** Results of the one step real-time RT-PCR, before selected tissues were fixed with formalin.

***RNA quality measurement***

Quality of the extracted RNA was assessed using a spectrophotometer (DeNovix Inc., Wilmington, USA). The absorbances of samples were measured at 260 nm and 280 nm. Purity of the samples was determined with ratio of 260/280.

***Detection of PPRV by one step RT-PCR***

PPRV specific RNA was detected using one-step RT-PCR kit (Qiagen, Hilden, Germany). One step RT-PCR reaction mixture was prepared containing, 0.4 µM of each primer and 2.6 µl of the extracted RNA, in a final volume of 20 µl. One-step RT-PCR was performed with primers described by Forsyth and Barrett (1995) which amplify the 448 bp of the F gene of PPRV. Amplification conditions were performed according to previous report (Şevik and Sait, 2015) using a thermal cycler (Techne, UK). PCR products were assessed in 1.5% agarose gel stained with GelRed (Biotium, Fremont, CA, USA).

## RESULTS

PPRV-RNA was detected in 12 FFPE tissue samples by one-step RT-PCR. However, three FFPE tissue samples that had been found positive by one step real time RT-PCR were found negative by one-step RT-PCR.

Additionally, five FFPE tissue samples that had been found negative by one step real time RT-PCR were also found negative by one-step RT-PCR. Detailed one-step real time RT-PCR and RT-PCR results are shown in Table 1.

**Table 1.** Results of the one step RT-PCR with modified extraction method

| Sample No | Animal species | One step Real time RT-PCR Ct values before FFPE procedures | One step RT-PCR | |
|---|---|---|---|---|
| | | | Results | Product intensity |
| **1** | Lamb | 24.70 | Positive | Strong |
| **2** | Lamb | 39.20 | Negative | |
| **3** | Lamb | 32.40 | Positive | Weak |
| **4** | Kid | 24.62 | Positive | Strong |
| **5** | Sheep | 26.50 | Positive | Strong |
| **6** | Kid | 39.55 | Negative | |
| **7** | Lamb | 38.44 | Negative | |
| **8** | Lamb | 25.45 | Positive | Strong |
| **9** | Lamb | 27.54 | Positive | Strong |
| **10** | Lamb | 28.33 | Positive | Strong |
| **11** | Lamb | 31.85 | Positive | Weak |
| **12** | Sheep | 26.98 | Positive | Strong |
| **13** | Lamb | 30.96 | Positive | Weak |
| **14** | Lamb | 27.23 | Positive | Strong |
| **15** | Lamb | 32.96 | Positive | Weak |
| **16** | Lamb | - | Negative | |
| **17** | Kid | - | Negative | |
| **18** | Lamb | - | Negative | |
| **19** | Lamb | - | Negative | |
| **20** | Lamb | - | Negative | |
| **N.C** | Lamb | - | Negative | |

NC = Negative control

Purity of the extracted RNA analyses based on A260/280 ratio revealed that 8 samples had ratios around 2.0 whereas 4 samples had a ration 1.8-1.9. Additionally, three samples had a ration < 1.8.

Samples with Ct values between 24 and 28 had strong product intensity whereas samples with Ct values between 30 and 32 had weak product intensity (Figure 2). However, samples with Ct values between 38 and 39 had no PCR product on one step RT-PCR.

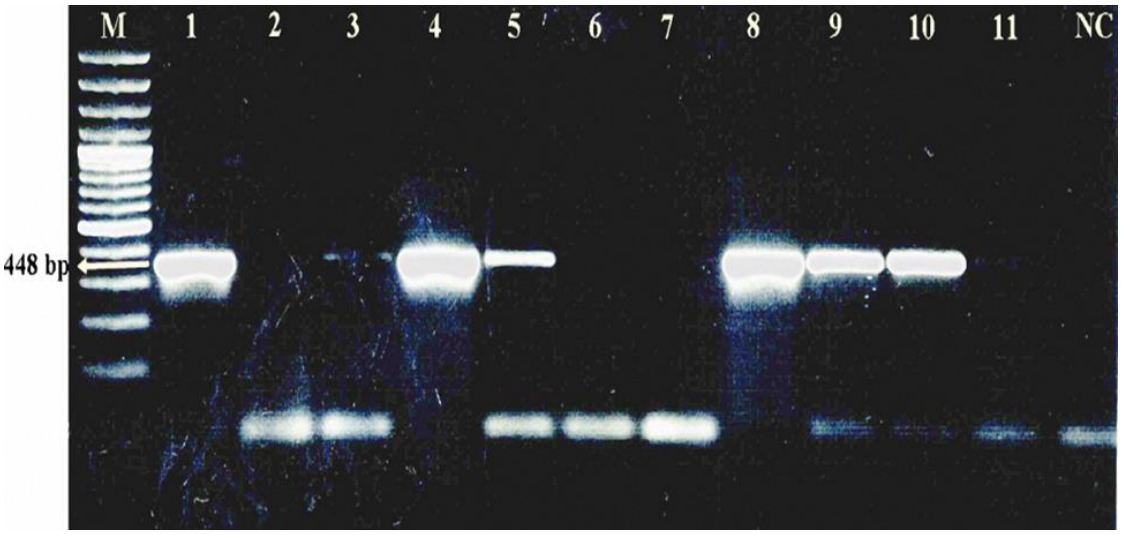


**Figure 2.** One step RT-PCR products based on F gene of PPRV. M: Marker (100 bp), Lane 1-11: Samples; Lane 3 and Lane 11 were weak positive; Lane 2, 6 and 7 were false negative results. NC: Negative control.

## DISCUSSION

PPR is one of the notifiable viral diseases of goats and sheep, and has been diagnosed in different regions of Turkey (OIE, 1999; Ozkul et al., 2002; Şevik and Sait, 2015). Different techniques can be used for confirmation of PPRV infection such as molecular techniques, virus isolation, imunocapture ELISA and agar gel immunodiffusion. Virus isolation is not commonly used for diagnosis of PPR because it is expensive and labour-intensive (Hudu et al., 2016). Molecular diagnostic techniques are commonly used currently to diagnose viral diseases. They are more sensitive than virus culture and provide a more rapid diagnosis of viral infections (van Elden et al., 2002). Furthermore, molecular techniques are recommended for confirmation of PPR suspected cases by OIE (OIE, 2022). Therefore, RT-PCR assays are commonly used in diagnostic laboratories.

RT-PCR methods are dependent upon high-quality nucleic acids extractions from samples (Browne et al., 2020). Fresh tissues and swab specimens are recommended for obtaining high quality RNA (Şevik, 2014). FFPE processing is an economical approach to storage of the specimens for extended periods of time, and widely used in research and diagnostics (Guo et al., 2017). Also, in some cases, it may not be possible to find fresh tissue samples. Therefore, FFPE tissue samples can be used for RNA isolation. However, extraction of amplifiable RNA from FFPE tissues is challenging because of nucleic acid degradation due to the fixation process (von Ahlfen et al., 2007). Therefore, special commercial kits have been developed for detection of nucleic acids from FFPE tissues. However, FFPE DNA/RNA purification kits are expensive than other extraction kits. In this study, a commercial kit which developed for total nucleic acid isolation from fresh tissues was modified, and it was evaluated for detection of PPRV from FFPE tissues. To improve RNA yields and purity and to degrade cross-linked RNAs, overnight proteinase K incubation period was added to the procedure of modified extraction method.

In the current study, PPRV specific RNA was detected in 12 of 15 lung tissue samples which were previously found positive by N gene based one step real time RT-PCR. Three positive samples, had been found positive by one step real time RT-PCR, were found negative by one-step RT-PCR. Furthermore, five FFPE tissue samples that had been found negative by one step real time RT-PCR method were also found negative by one-step RT-PCR. The difference between results of one step RT-PCR and one step real time RT-PCR can be explained by extraction method, analytical sensitivities of the methods and RNA quality of the three samples that were found negative by RT-PCR method. Because these three samples that had been found positive by one step real time RT-PCR had high Ct values when compared other samples (Table 1). It has been reported that viral load is inversely related with Ct values, a high Ct value indicating a low viral load (Bonacorsi et al., 2021). Furthermore, these three samples that were found negative by one step RT-PCR had an A260/280 ratio under 1.8. It has been reported that ratios under 1.8 could indicate the presence phenol, proteins and other contaminants (Glasel, 1995). The purity of the RNA is very important for RT-PCR (Banko et al., 2021). Therefore, these false negative results can be explained by low purity of the samples.

Conventional RT-PCR has lower sensitivity compared with real time RT-PCR. Therefore, real time RT-PCR can detect lower viral loads which were below the detection limit of the one-step RT-PCR (Banko et al., 2021; Ramamurthy et al., 2011). Furthermore, it has been reported that formalin fixation causes RNA degradation, poly-A tail damage and RNA

modification by adding methylol groups (-$CH_2OH$) which are reduce PCR efficiency (Evers et al., 2011; Masuda et al., 1999). Therefore, these modifications because of formalin fixation may lead to false negative results.

In this study, PPRV F gene sequences were successfully amplified by one step RT-PCR assay using extracted RNA from FFPE lung tissues. Limited number of studies has been performed for detection of morbillivirus RNA from FFPE tissues (Liang et al., 2012; Seimon et al., 2013). To detect canine distemper virus RNA in FFPE tissues, Liang et al. (2012) used in situ hybridization (ISH) whereas Seimon et al. (2013) used RT-PCR and ISH. Furthermore, detection of PPRV specific RNA in FFPE tissues by real time RT-PCR has been reported (Kihu et al., 2015). Detection of PPRV RNA in FFPE samples allow to use FFPE tissues in the confirmation of PPR in laboratories where fresh tissues are not available for RNA extraction.

In this study, one step RT-PCR assay was performed with primers targeting F gene of PPRV. Primers targeting nucleoprotein (N) gene of the PPRV were also chosen for detection of PPRV in FFPE tissues (Kihu et al., 2015). Therefore, results of the studies suggest that both of the primers can be used for detection of PPRV in FFPE tissues.

## CONCLUSION

In conclusion, results of the current study show that modified extraction method could be used for PPRV RNA extraction from FFPE tissues which had been stored for 2 years. However, viral load in FFPE tissues should be considered when using this modified extraction method. This provides an opportunity to field veterinary laboratories that may lack cold storage facilities to keep fresh pathological samples for PPR diagnoses.

## ACKNOWLEDGMENT

The part of the study was presented as an oral presentation in the 4th International Conference on Medical and Health Sciences in Burdur, Turkey, 19-20 March, 2022.

Ethical approval: This study data was used with the permission of General Directorate of Food and Control dated 13.11.2017 and numbered E.2852005.

## REFERENCES

**Abraham, G., Sintayehu, A., Libeau, G., Albina, E., Roger, F., Laekemariam, Y., Abayneh, D., & Awoke, K.M. (2005).** Antibody seroprevalences against peste des petits ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. *Preventive veterinary medicine*, *70*(1-2), 51-57. https://doi.org/ 10. 1016 / j.prevetmed.2005.02.011.

**Abubakar, M., Rajput, Z.I., Arshed, M.J., Sarwar, G., & Ali, Q. (2011).** Evidence of peste des petits ruminants virus (PPRV) infection in Sindh Ibex (Capra aegagrus blythi) in Pakistan as confirmed by detection of antigen and antibody. *Tropical animal health and production*, *43*(4), 745-747. https://doi.org/10.1007/s11250-010-9776-y.

**Bailey, D., Banyard, A., Dash, P., Ozkul, A., & Barrett, T. (2005).** Full genome sequence of peste des petits ruminants virus, a member of the Morbillivirus genus. *Virus research*, *110*(1-2), 119-124. https://doi.org/10.1016/j.virusres.2005.01.013.

**Banko, A., Petrovic, G., Miljanovic, D., Loncar, A., Vukcevic, M., Despot, D., & Cirkovic, A. (2021).** Comparison and Sensitivity Evaluation of Three Different Commercial Real-Time Quantitative PCR Kits for SARS-CoV-2 Detection. *Viruses*, *13*(7), 1321. https://doi.org/10.3390/v13071321.

**Banyard, A.C., Wang, Z., & Parida, S. (2014).** Peste des petits ruminants virus, eastern Asia. *Emerging infectious diseases*, *20*(12), 2176-2178. https://doi.org/10.3201/eid2012.140907.

**Batten, C.A., Banyard, A.C., King, D.P., Henstock, M.R., Edwards, L., Sanders, A., Buczkowski, H., Oura, C.C., & Barrett, T. (2011).** A real time RT-PCR assay for the specific detection of Peste des petits ruminants virus. *Journal of virological methods*, *171*(2), 401-404. https://doi.org/10.1016/j.jviromet. 2010.11.022.

**Bonacorsi, S., Visseaux, B., Bouzid, D., Pareja, J., Rao, S.N., Manissero, D., Hansen. G., & Vila, J. (2021).** Systematic Review on the Correlation of Quantitative PCR Cycle Threshold Values of Gastrointestinal Pathogens With Patient Clinical Presentation and Outcomes. *Frontiers in medicine*, *8*, 711809. https://doi.org/10.3389/fmed.2021.711809.

**Browne, D.J., Brady, J.L., Waardenberg, A.J., Loiseau, C., & Doolan, D.L. (2020).** An Analytically and Diagnostically Sensitive RNA Extraction and RT-qPCR Protocol for Peripheral Blood Mononuclear

Cells. *Frontiers in immunology*, *11*, 402. https://doi.org/10.3389/fimmu.2020.00402.

**Couacy-Hymann, E., Bodjo, S.C., Danho, T., Koffi, M.Y., Libeau, G., & Diallo, A. (2007).** Early detection of viral excretion from experimentally infected goats with peste-des-petits ruminants virus. *Preventive veterinary medicine*, *78*(1), 85-88. https://doi.org/10.1016/j.prevetmed.2006.09.003.

**Couacy-Hymann, E. (2015).** Current Advances in Genome Detection of Peste des Petits Ruminants Virus. In M. Munir (Eds.), *Peste des Petits Ruminants Virus*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-45165-6_9.

**Dou, Y., Liang, Z., Prajapati, M., Zhang, R., Li, Y., & Zhang, Z. (2020).** Expanding Diversity of Susceptible Hosts in Peste Des Petits Ruminants Virus Infection and Its Potential Mechanism Beyond. *Frontiers in veterinary science*, *7*, 66. https://doi.org/10.3389/fvets.2020.00066.

**Evers, D.L., He, J., Kim, Y.H., Mason. J.T., & O'Leary, T.J. (2011).** Paraffin embedding contributes to RNA aggregation, reduced RNA yield, and low RNA quality. *The Journal of Molecular Diagnostics*, *13*(6), 687-694. https://doi.org/10.1016/j.jmoldx.2011.06.007.

**Fakri, F.Z., Bamouh, Z., Jazouli, M., Omari, Tadlaoui, K., & Elharrak, M. (2019).** Experimental infection of dromedary camels with virulent virus of Peste des Petits Ruminants. *Veterinary microbiology*, *235*, 195-198. https://doi.org/10.1016/j.vetmic.2019.07.004.

**Forsyth, M.A., & Barrett, T. (1995).** Evaluation of polymerase chain reaction for the detection and characterisation of rinderpest and peste des petits ruminants viruses for epidemiological studies. *Virus research*, *39*(2-3), 151-163. https://doi.org/10.1016/0168-1702(95)00076-3.

**Gibbs, E.P., Taylor, W.P., Lawman, M.J., & Bryant, J. (1979).** Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. *Intervirology*, *11*(5), 268-274. https://doi.org/10.1159/000149044.

**Glasel, J.A. (1995).** Validity of nucleic acid purities monitored by 260nm/280nm absorbance ratios. *Biotechniques*, *18*(1), 62-63.

**Guo, Y., Han, L., & Sheng, Q. (2017).** Recent Advances in High Throughput Sequencing Analysis. *International Journal of Genomics*, *2017*, 2454780. https://doi.org/10.1155/2017/2454780.

**Hudu, S.A., Alshrari, A.S., Syahida, A., & Sekawi, Z. (2016).** Cell Culture, Technology: Enhancing the Culture of Diagnosing Human Diseases. *Journal of clinical and diagnostic research*, *10*(3), 1-5. https://doi.org/10.7860/JCDR/2016/15837.7460.

**ICTV. (2021, February 09).** *Taxonomic Information.* https://talk.ictvonline.org/taxonomy/

**Kerur, N., Jhala, M.K., & Joshi, C.G. (2008).** Genetic characterization of Indian peste des petits ruminants virus (PPRV) by sequencing and phylogenetic analysis of fusion protein and nucleoprotein gene segments. *Research in veterinary science*, *85*(1), 176-183. https://doi.org/10.1016/j.rvsc.2007.07.007.

**Kihu, S.M., Gitao, G.C., Bebora, L.C., Njenga, M.J., Wairire, G.G., Maingi, N., Wahome, R.G., Oyugi, J.O., & Lutomia, E. (2015).** Detection of peste des petits ruminants virus in formalin-fixed tissues. *Tropical animal health and production*, *47*(1), 247-249. https://doi.org/10.1007/s11250-014-0707-1.

**Li, J., Li, L., Wu, X., Liu, F., Zou, Y., Wang, Q., Liu, C., Bao, J., Wang, W., Ma, W., Lin, H., Huang, J., Zheng, X., & Wang, Z. (2017).** Diagnosis of Peste des Petits Ruminants in Wild and Domestic Animals in Xinjiang, China, 2013-2016. *Transboundary and emerging diseases*, *64*(6), 43-47. https://doi.org/10.1111/tbed.12600.

**Liang, C.T., Chueh, L.L., Pang, V.F., Lee, K.H., Liang, S.C., Lee, C.C., & Liu, C.H. (2012).** Improving Detection of Canine Distemper Virus in Formalin-Fixed, Paraffin-Embedded Tissues: Using in situ Hybridization with Integrated Optical Density to Give a Semi-Quantitative Assessment. *International Journal of Applied Research in Veterinary Medicine*, *4*(10), 335-343.

**Mahapatra, M., Sayalel, K., Muniraju, M., Eblate, E., Fyumagwa, R., Shilinde, L., Mdaki, M., Keyyu, J., Parida, S., & Kock, R. (2015).** Spillover of Peste des Petits Ruminants Virus from Domestic to Wild Ruminants in the Serengeti Ecosystem, Tanzania. *Emerging infectious diseases*, *21*(12), 2230-2234. https://doi.org/10.3201/eid2112.150223.

**Mapaco, L., Monjane, I., Fafetine, J., Arone, D., Caron, A., Chilundo, A., Quembo, C., Carrilho, M.D.C., Nhabomba, V., Zohari, S., & Achá, S. (2019).** Peste des Petits Ruminants Virus Surveillance in Domestic Small Ruminants, Mozambique (2015 and 2017). *Frontiers in veterinary science*, *6*, 370. https://doi.org/10.3389/fvets.2019.00370.

**Masuda, N., Ohnishi, T., Kawamoto, S., Monden, M., & Okubo, K. (1999).** Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples. *Nucleic acids research*, *27*(22), 4436-4443. https://doi.org/10.1093/nar/27.22.4436.

**OIE. (1999, July 15).** *OIE disease information*. https://www.oie.int/en/what-we-do/animal-health-and-welfare/disease-data-collection/world-animal-health-information-system/

**OIE. (2022, February 09).** *Peste des petits ruminants*. https://www.oie.int/en/disease/peste-des-petitsruminants/#:~:text=Peste%20des%20petits%20ruminants%20(PPR)%20is%20a%20contagious%20transboundary%20disease,rural%20communities%2C%20notably%20of%20women

**Ozkul, A., Akca, Y., Alkan, F., Barrett, T., Karaoglu, T., Dagalp, S.B., Anderson, J., Yesilbag, K., Cokcaliskan, C., Gencay, A., & Burgu, I. (2022).** Prevalence, distribution, and host range of Peste des petits ruminants virus, Turkey. Emerging infectious diseases, *8*(7), 708-712. https://doi.org/10.3201/eid0807.010471.

**Parida, S., Selvaraj, M., Gubbins, S., Pope, R., Banyard, A., & Mahapatra, M. (2019).** Quantifying Levels of Peste Des Petits Ruminants (PPR) Virus in Excretions from Experimentally Infected Goats and

Its Importance for Nascent PPR Eradication Programme. *Viruses*, *11*(3):249. https://doi.org/10.3390/v11030249.

**Ramamurthy, M., Alexander, M., Aaron, S., Kannangai, R., Ravi, V., Sridharan, G., & Abraham, A.M. (2011).** Comparison of a conventional polymerase chain reaction with real-time polymerase chain reaction for the detection of neurotropic viruses in cerebrospinal fluid samples. *Indian Journal of Medical Microbiology*, *29*(2), 102-109. https://doi.org/10.4103/0255-0857.81777.

**Seimon, T.A., Miquelle, D.G., Chang, T.Y., Newton, A.L., Korotkova, I., Ivanchuk, G., Lyubchenko, E., Tupikov, A., Slabe, E., & McAloose, D. (2013).** Canine distemper virus: an emerging disease in wild endangered Amur tigers (Panthera tigris altaica). *mBio*, *4*(4), e00410-413. https://doi.org/10.1128/mBio.00410-13.

**Şevik, M. (2014).** Molecular Detection of Peste des Petits Ruminants Virus from Different Organs/Tissues of Naturally Infected Animals. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, *20*(1), 165-168. https://doi.org/10.9775/kvfd.2013.

**Şevik, M., & Sait, A. (2015).** Genetic characterization of peste des petits ruminants virus, Turkey, 2009-2013. *Research journal of veterinary sciences*, *101*, 187-95. https://doi.org/10.1016/j.rvsc.2015.05.005.

**Van Elden, L.J., van Kraaij, M.G., Nijhuis, M., Hendriksen, K.A., Dekker, A.W., Rozenberg-Arska, M., & van Loon, A.M. (2002).** Polymerase chain reaction is more sensitive than viral culture and antigen testing for the detection of respiratory viruses in adults with hematological cancer and pneumonia. *Clinical infectious diseases*, *34*(2), 177-183. https://doi.org/10.1086/338238.

**Von Ahlfen, S., Missel, A., Bendrat, K., & Schlumpberger, M. (2007).** Determinants of RNA quality from FFPE samples. *PLoS One*, *2*(12), e1261. https://doi.org/10.1371/journal.pone.0001261.