Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

The first discovery of Chaphamaparvovirus in sheep with encephalitis and anemia

Ensefalitli ve anemili koyunda Chaphamaparvovirüs'ün ilk keşfi

Eda ALTAN[1,2] (ID), Gilberto SABINO-SANTOS JR[1,2] (ID), Patricia PESAVENTO[3] (ID), Eric DELWART[1,2] (ID)

ABSTRACT

Objective: Parvoviruses have been shown to exist in sheep since 1987. To this date there are only three reports pertaining to the existence parvoviruses in sheep. The first reported parvovirus study did not provide genomic information whereas the latter two belonged to the tetraparvovirus and copiparvovirus genera. This study focused on discovering the possible reasons of encephalitis and anemia in a dead sheep whose tissue samples were submitted to our laboratory.

Methods: In the present study next-generation sequencing (NGS) was utilized. Nextera™ XT Sample Preparation Kit was used to generate a library for Illumina MiSeq using dual barcoding. An in-house pipeline was used for analyzing raw data generated from Miseq. Several software were used to create in house-pipeline to trim sequences and de novo assembly. For the alignment genome and phylogenetic tree Geneious and MEGA X software were used.

Results: A novel ovine chaphamaparvovirus and pestivirus D both were characterized simultaneously. Although chaphamaparvoviruses had had been reported in various animals, this is the first time they have been reported in sheep. PCR analyses confirmed the presence of chaphamaparvovirus in multiple tissues. The partial nonstructural protein (NS1) and the complete capsid

ÖZET

Amaç: Parvovirüsler koyunlarda ilk olarak 1987 yılında tespit edilmiştir. Günümüze kadar koyunlarda parvovirüslerin varlığına ilişkin sadece üç rapor vardır. İlk bildirilen parvovirüs çalışması genomik bilgi sağlamazken, son ikisi tetraparvovirus ve copiparvovirus cinslerine aittir. Bu çalışmada doku örnekleri laboratuvarımıza gönderilen sebebi belli olmayan ensefalit ve anemi tespit edilen ölü bir koyunda olası nedenleri keşfetmeye odaklanılmıştır.

Yöntem: Bu çalışmada yeni nesil dizileme (NGS) kullanılmıştır. Nextera™ XT Numune Hazırlama Kiti, ikili barkod kullanarak Illumina MiSeq platformu için bir kitaplık oluşturmada kullanıldı. Miseq'ten üretilen ham verileri analiz etmek için şirket içi bir data analiz altyapısı kullanıldı. Okuma dizilerini kırpmak ve de novo bağlama analizi için çeşitli yazılımlar kullanıldı. Genomun hizalama işlemi ve filogenetik ağaç için Geneious ve MEGA X yazılımından faydalanıldı.

Bulgular: Çalışmada yeni bir küçükbaş hayvan chaphamaparvovirüsü ve pestivirus D aynı anda karakterize edildi. Chaphamaparvovirüsler çeşitli hayvanlarda bildirilmiş olmasına rağmen koyunlarda ilk kez rapor edilmektedir. PCR analizleri, birçok dokuda chaphamaparvovirus varlığını doğruladı. Kısmi yapısal olmayan protein (NS1) ve tam kapsid proteinleri (VP1)

[1]Vitalant Research Institute, San Francisco, USA
[2]University of California, Department of Laboratory Medicine, San Francisco, USA
[3]AUC Davis School of Veterinary Medicine, Department of Pathology, Microbiology and Immunology

İletişim / Corresponding Author : Eda ALTAN
270 Masonic Vitalant Research Institute, San Francisco, CA 94118 - USA
E-posta / E-mail : edaltan@hotmail.com

Geliş Tarihi / Received : 16.06.2021
Kabul Tarihi / Accepted : 30.06.2021

DOI ID : 10.5505/TurkHijyen.2021.07348

Altan E, Sabino-Santos G, Pesavento P, Delwart E.The first discovery of Chaphamaparvovirus in sheep with encephalitis and anemia.
Turk Hij Den Biyol Derg, 2021; 78(3): 343 - 350

proteins (VP1) protein sequences displayed the closest amino acid identity of 49% and 69%, respectively, to the proteins of a non-human primate chapparvovirus from *Macaca fascicularis*.

Conclusion: This ovine parvovirus is the fourth parvovirus and the first chaphamaparvovirus reported in sheep. Both chaphamaparvovirus and pestivirus were shown to co-exist simultaneously in sheep. The role of this dual virus infection in the disease signs of this sheep remains to be determined. This study will shed light on future chaphamaparvovirus studies in sheep.

Key Words: Next-generation sequencing, Parvovirus, Chaphamaparvovirus, ovine

protein dizileri, insan olmayan bir primat *Macaca fascicularis*'te bulunan chapparvovirüsün proteinlerine sırasıyla %49 ve %69'luk en yakın amino asit özdeşliğini sergiledi.

Sonuç: Bu küçükbaş hayvan parvovirüsü koyunlarda bildirilen dördüncü parvovirüs ve ilk chaphamaparvovirustur. Hem chaphamaparvovirus hem de pestivirüs'ün koyunlarda aynı anda birlikte var olduğu gösterilmiştir. Bu koyunun hastalık belirtilerinde bu ikili virüs enfeksiyonunun rolü henüz belirlenmemiştir. Bu çalışma ileride koyunlarda yapılacak olan chaphamaparvovirus çalışmalarına ışık tutacak niteliktedir.

Anahtar Kelimeler: Yeni nesil dizileme, Parvovirus, Chaphamaparvovirus, küçükbaş hayvan

INTRODUCTION

The genomes of members of the *Parvoviridae* family members are ssDNA and 4-6.3 kb in size with two to four recognized open reading frames (ORFs). *Parvoviridae* family is now classified into 3 different subfamilies: recently added *Hamaparvovirinae* capable of infecting either invertebrate or vertebrate, Parvovirinae infecting vertebrate hosts, Densovirinae infecting invertebrate hosts (1). The subfamily *Hamaparvovirinae* can be divided into five genera. Members of four of them infect invertebrates (*Penstylhamaparvovirus*, *Brevihamaparvovirus*, *Hepanhamaparvovirus*, *Ichthamaparvovirus*) while members of *Chaphamaparvovirus* genus infect vertebrates (1). *Chaphamaparvovirus* genus members have been defined in multiple animal species including human (2), dogs (3, 4), cats (4), bear (5), wild birds (6), red-crowned cranes (7), turkeys and chickens (8, 9), reptile (10), bats (11, 12), macaques (13, 14), pigs (15), tasmanian devils (16), fish (17), and rats (18, 19). Disease association has not been established in most species, however a murine *Chaphamaparvovirus*, named Mouse kidney parvovirus causes chronic tubulointerstitial nephritis in both immonocompromised and immunocompetent laboratory mice (20, 21). Another *Chaphamaparvovirus* has been associated with massive mortality in tilapia fish. Chapahamaparvoviruses have also been associated with diarrhea in dogs (3) and cats (4).

Small ruminants are important to the livelihoods of farmers around the world, and food security is important for both their milk and meat products consumed by the public. Diverse viruses including pestivirus (Border disease), bunyavirus (Rift valley fever), pox virus (Contagious ecthyma, Sheep and goat pox), orbivirus (Bluetongue), retrovirus (Pulmonary adenomatosis-Jaagsiekte, Driving sickness), lentivirus (Ovine progressive interstitial pneumonia) and orthonairovirus (Nairobi sheep disease) are known to cause pathogenic infections in sheep (22). The first parvovirus in sheep was reported in 1987

and named ovine parvovirus but detailed information including its genome is not available (23). In 2011, a parvovirus (ovine hokovirus 1) in the liver and spleen of a sheep was sequenced, and classified into the Ungulate tetraparvovirus four species (24). This virus has not (yet) been associated with clinical symptoms (24). Recently, another sheep parvovirus belongs to copiparvovirus genera was reported from Brazil (25).

Here, we used deep sequencing to evaluate the virome of multiple tissues from a sheep that had both anemia and encephalitis of unknown origin. A novel *Chaphamaparvovirus* genome was characterized and its presence in multiple tissues confirmed by PCR.

MATERIAL and METHOD

The animals were submitted with owner consent for routine necropsy to the Anatomic Pathology Service of the Veterinary Medical Teaching Hospital at the School of Veterinary Medicine at UC Davis. Lung, spleen and liver samples were collected at the time of routine necropsy from a sheep with a clinical evaluation of encephalopathy and anemia. The main histologic findings were myelitis (spinal cord white matter degeneration), crusting skin lesions, lymphocytic perivascular cuffing in multiple tissues, gastrointestinal erosion, and proliferative colitis.

Viral Discovery

A tissue pool of lung, spleen and liver tissues stored at -80˚C from index sheep case was thawed and a hand-held rotor was used with 10X volume of phosphate-buffered saline for tissue homogenization. Homogenate was then rapidly frozen and thawed on dry ice 5 times. Centrifugation (10 min, 15 000 x g) used to collect the supernatant (400 ml). The supernatant was filtered through a 0.45 mm centrifugal filter (Millipore). The filtrate was treated with enzyme cocktail at 37˚C for 90 minutes for viral enrichment (26). The MagMAX Viral RNA Isolation kit (Ambion) protocol was used with small modification (without carrier RNA) for nucleic acid extraction. SuperScript™ III Reverse Transcriptase (invitrogen) was used for reverse transcription step and than random PCR reaction was performed with random primer (26). Nextera™ XT Sample Preparation Kit (Illumina) was used to generate a library for Illumina MiSeq (2 × 250 bases) using dual barcoding (26). An in-house pipeline was used for analyzing raw data generated from Miseq. Several software were used to create in house-pipeline to trim sequences and de novo assembly (27, 28). BLASTx (version 2.2.7). BLASTx (version 2.2.7) was used to analyze singlets and contigs for similarity to all viral protein sequences available in GenBank's virus RefSeq and non-redundant database using an E-value cutoff of 0.001. DIAMOND (version 0.9.6) was then used to compare initial viral hits to all protein sequences in NR and were retained only when the top hit was to a sequence annotated as viral. The Geneious R10 program was used for alignments, translation to amino acid, and ORF finding. The amino acid (aa) pairwise alignments of Parvoviruses Non-structural protein 1 (NS1) and Capsid protein (VP1) and pestivirus were performed by the Genious using the in-built MAFFT algorithm. The amino acid phylogenetic trees were constructed using the Maximum likelihood method with two substitution models: Le Gascuel 2008 model based with gamma distributed (G+) for NS1 and VP1 and D polyprotein in MEGA software version X (29, 30).

PCR detection

To confirm that the parvoviral genome was present in tissues from this animal, PCR primers Ovichap_F (5'- GCAGAGAGGACAACAACCCATA-3') and Ovichap_R (5'- ACCATTTCCCCATTGCTCTCAT-3') were used to amplify a 471 bp size of ovine chaphamaparvovirus NS1 region. The mixture was placed in a thermal cycler (Biorad, Chromo-4) and the polymerase activated, by incubation at 95˚C for 5 min. Cycling conditions were 95˚C for 30 s, 56˚C for 30 s, and 72˚C for 1 min for 35 cycles, a final extension at 72˚C for 10 min. Bands of expected size were isolated and extracted and Sanger sequenced.

RESULTS

The lung, liver and splenic tissue of a neurologic, anemic sheep were pooled and processed for viral metagenomics. Virus like particle (VLP)-associated DNA and RNA fragments were processed for sequencing following enrichment and random amplification. Illumina MiSeq platform was used for library generation (materials and methods) and resulting raw sequence data can be accessed at NCBI's GenBank with accession number SRP13198876. BLASTx analyses of the fragments yielded to chaphamaparvovirus proteins with E score ranging from $3.44e^{-34}$ to 4.64^{e-11} including a 2603 bases contigs. Also detected were 7321 reads matching Pestivirus D yielding a complete polyprotein encoding sequence. The chaphamaparvovirus contigs were used to design PCR primers. Lung, liver, and splenic tissues were separately extracted and individually tested by PCR and Sanger sequencing and all tissues contained detectable parvoviral genome.

A partial genome of a virus we callled ovine chaphamaparvovirus (GenBank accession number MW344047) of 2603 nucleotides was generated. The genome was 42.8% G+C with a distribution of 37.1% A, 20.1% T, 20.6% G and 22.1% C. The expected ATP- or GTP-binding Walker A loop motif (GxxxxGKT/S; GPSNTGKS) and Walker B motif (EE) were found in NS1(31).

The partial nonstructural protein (NS1) and the complete capsid proteins (VP1) proteins had closest aa identity of 49% and 69%, to the corresponding proteins of Macaca fascicularis chapparvovirus (MN3122221) (Figure 1).

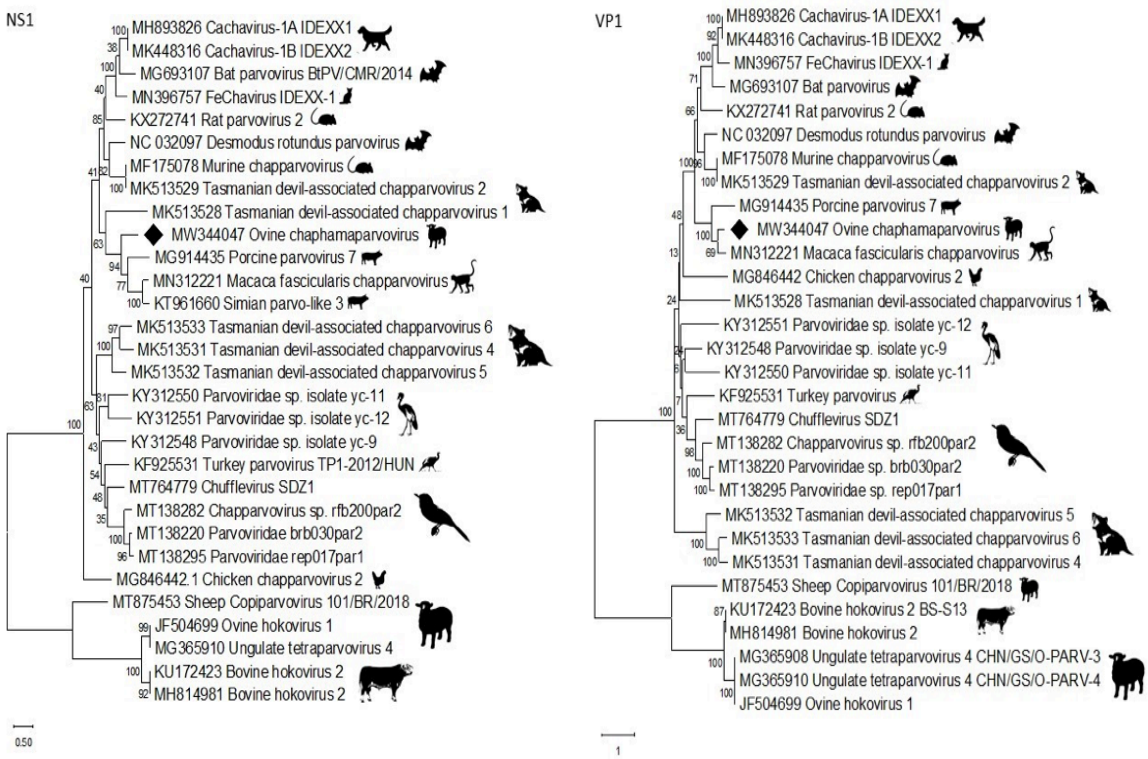

Figure 1. Phylogenetic trees (A; NS1 and B; VP1) were constructed using the Maximum likelihood method with two substitution models: Le_Gascule_2008 model (LG) with Freqs and gamma distributed, invariant sites (G + I) model MEGA software version X.

Protein based phylogenetic trees of this new chaphamaparvovirus and of the pestivirus D strain were constructed using Maximum likelihood method (see materials and methods). The novel ovine chaphamaparvovirus proteins both fell within the *Chaphamaparvovirus* genus.

The complete pestivirus D polyprotein coding region also detected in this sheep tissue pool was assembled (MT108680) showing best amino acid identity of 94% to Border disease virus strain: BD31 from the USA (U70263), FNK2012-1 from Japan (AB897785), and strain Coos Bay 5 from USA (KJ463422) (Figure 2).

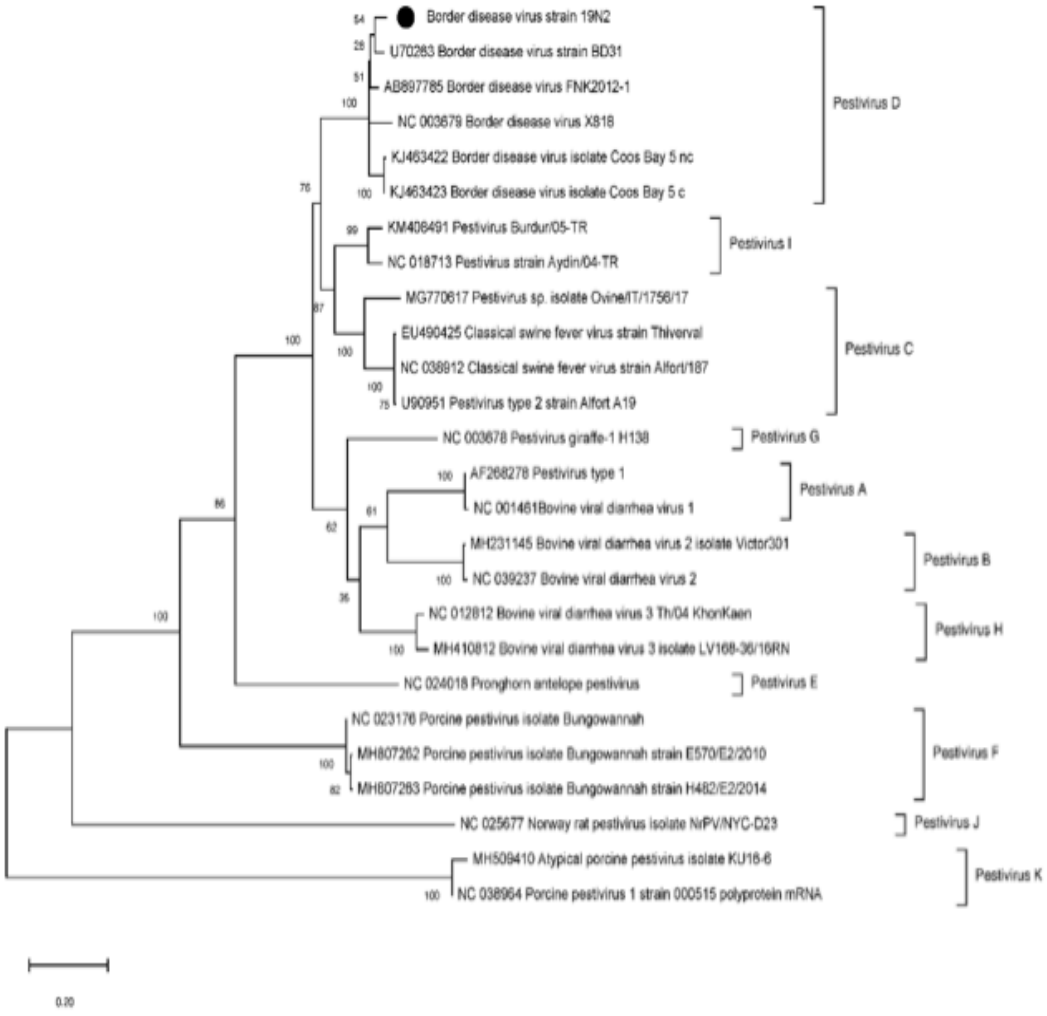

Figure 2. PThe complete pestivirus D polyprotein phylogenetic tree.

DISCUSSION and CONCLUSION

We describe here the first ovine chaphamaparvovirus. The viral genome encoded typical genome organization and ORFs. Based on ICTV criteria (1) the low level of sequence identity (<85% identity in NS1) between the ovine chaphamaparvovirus genome and those of the other known chaphamaparvoviruses indicates membership in a distinct viral species. We propose the name ungulate chaphamaparvovirus 2 as the species name following ungulate chaphamaparvovirus 1 containing porcine parvovirus 7 (KU5637330), the only member of that species to date (15).

Pestivirus D (Border disease virus) first discovered in 1959 and has been reported world wide in both sheep and goats. Acute infections of Border disease generally are associated with a mild leukopenia and slight-fever. The main significant, but less common, clinical sequelae is abortion (32, 33). The disease signs exhibited by this sheep are not consistent with those typically associated with Border disease virus.

Viral encephalitis and anemia are known signs for lentiviral infections in sheep such as maedi-visna disease. Small ruminant lentiviruses cause chronic inflammatory and degenerative lesions in the brain, lungs, joints and mammary glands (34, 35). The main findings include spinal cord white matter degeneration, crusting skin lesions, and lymphocytic perivascular cuffing in a variety of organs (36). Although the symptoms were similar to those of lentiviral disease, no lentiviral read was observed in the viral metagenomics of the affected sheep's tissues.

The role of novel ovine chaphamaparvovirus infection in the clinical signs and pathological lesions of this sheep remains to be determined. Availability of the chaphamaparvovirus genome will facilitate detection in sick or healthy sheep in order to further examine a potential pathogenic role.

ACKNOWLEDGMENTS

We acknowledge the support from Vitalant Research Institute (formerly Blood Systems Research Institute).

ETHICS COMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Penzes JJ, Soderlund-Venermo M, Canuti M, Eis-Hubinger AM, Hughes J, Cotmore SF, et al. Reorganizing the family Parvoviridae: a revised taxonomy independent of the canonical approach based on host association. Arch Virol. 2020;165(9):2133-46.

2. Fahsbender E, Charlys da-Costa A, Elise Gill D, Augusto de Padua Milagres F, Brustulin R, Julio Costa Monteiro F, et al. Plasma virome of 781 Brazilians with unexplained symptoms of arbovirus infection include a novel parvovirus and densovirus. PLoS One. 2020;15(3):e0229993.

3. Fahsbender E, Altan E, Seguin MA, Young P, Estrada M, Leutenegger C, et al. Chapparvovirus DNA found in 4% of dogs with diarrhea. Viruses. 2019;11(5).

4. Li Y, Gordon E, Idle A, Altan E, Seguin MA, Estrada M, et al. Virome of a feline outbreak of diarrhea and vomiting includes bocaviruses and a novel chapparvovirus. Viruses. 2020;12(5).

5. Alex CE, Fahsbender E, Altan E, Bildfell R, Wolff P, Jin L, et al. Viruses in unexplained encephalitis cases in American black bears (*Ursus americanus*). PLoS One. 2020;15(12):e0244056.

6. Altan E KS, Stalis I, Li Y, Deng X, Delwart E. Novel chaphamaparvovirus in a paradise tanager songbird with encephalitis, neuritis, and ventriculitis during the process for publish. 2021.

7. Wang Y, Yang S, Liu D, Zhou C, Li W, Lin Y, et al. The fecal virome of red-crowned cranes. Arch Virol. 2019;164(1):3-16.

8. Lima DA, Cibulski SP, Tochetto C, Varela APM, Finkler F, Teixeira TF, et al. The intestinal virome of malabsorption syndrome-affected and unaffected broilers through shotgun metagenomics. Virus Res. 2019;261:9-20.

9. Reuter G, Boros A, Delwart E, Pankovics P. Novel circular single-stranded DNA virus from turkey faeces. Arch Virol. 2014;159(8):2161-4.

10. Chang WS, Li CX, Hall J, Eden JS, Hyndman TH, Holmes EC, et al. Meta-transcriptomic discovery of a divergent circovirus and a chaphamaparvovirus in captive reptiles with proliferative respiratory syndrome. Viruses. 2020;12(10).

11. Baker KS, Leggett RM, Bexfield NH, Alston M, Daly G, Todd S, et al. Metagenomic study of the viruses of African straw-coloured fruit bats: detection of a chiropteran poxvirus and isolation of a novel adenovirus. Virology. 2013;441(2):95-106.

12. Yinda CK, Ghogomu SM, Conceicao-Neto N, Beller L, Deboutte W, Vanhulle E, et al. Cameroonian fruit bats harbor divergent viruses, including rotavirus H, bastroviruses, and picobirnaviruses using an alternative genetic code. Virus Evol. 2018;4(1):vey008.

13. Kapusinszky B, Ardeshir A, Mulvaney U, Deng X, Delwart E. Case-control comparison of enteric viromes in captive rhesus macaques with acute or idiopathic chronic diarrhea. J Virol. 2017;91(18).

14. Sawaswong V, Fahsbender E, Altan E, Kemthong T, Deng X, Malaivijitnond S, et al. High Diversity and Novel Enteric Viruses in Fecal Viromes of Healthy Wild and Captive Thai Cynomolgus Macaques (*Macaca fascicularis*). Viruses. 2019;11(10).

15. Palinski RM, Mitra N, Hause BM. Discovery of a novel Parvovirinae virus, porcine parvovirus 7, by metagenomic sequencing of porcine rectal swabs. Virus Genes. 2016;52(4):564-7.

16. Chong R, Shi M, Grueber CE, Holmes EC, Hogg CJ, Belov K, et al. Fecal viral diversity of captive and wild tasmanian devils characterized using virion-enriched metagenomics and metatranscriptomics. J Virol. 2019;93(11).

17. Du J, Wang W, Chan JF, Wang G, Huang Y, Yi Y, et al. Identification of a novel ichthyic parvovirus in marine species in Hainan Island, China. Front Microbiol. 2019;10:2815.

18. Williams SH, Che X, Garcia JA, Klena JD, Lee B, Muller D, et al. Viral Diversity of House Mice in New York City. mBio. 2018;9(2).

19. Yang S, Liu Z, Wang Y, Li W, Fu X, Lin Y, et al. A novel rodent chapparvovirus in feces of wild rats. Virol J. 2016;13:133.

20. Edmondson EF, Hsieh WT, Kramer JA, Breed MW, Roelke-Parker ME, Stephens-Devalle J, et al. Naturally acquired mouse kidney parvovirus infection produces a persistent interstitial nephritis in immunocompetent laboratory mice. Vet Pathol. 2020;57(6):915-25.

21. Roediger B, Lee Q, Tikoo S, Cobbin JCA, Henderson JM, Jormakka M, et al. An atypical parvovirus drives Chronic tubulointerstitial nephropathy and kidney fibrosis. Cell. 2018;175(2):530-43 e24.

22. Murphy FA. Veterinary virology. 3rd ed. San Diego: Academic Press; 1999. x, 629 p. p.

23. Chappuis G, Soulier M, Nettleton PF. Ovine parvovirus infection in Scottish sheep. Vet Rec. 1987;121(8):182.

24. Tse H, Tsoi HW, Teng JL, Chen XC, Liu H, Zhou B, et al. Discovery and genomic characterization of a novel ovine partetravirus and a new genotype of bovine partetravirus. PLoS One. 2011;6(9):e25619.

25. Mosena ACS, da Silva MS, Lorenzett MP, Cibulski SP, Weber MN, Budaszewski RF, et al. A new highly divergent copiparvovirus in sheep. Arch Virol. 2021;166(5):1517-20.

26. Li L, Deng X, Mee ET, Collot-Teixeira S, Anderson R, Schepelmann S, et al. Comparing viral metagenomics methods using a highly multiplexed human viral pathogens reagent. J Virol Methods. 2015;213:139-46.

27. Deng X, Naccache SN, Ng T, Federman S, Li L, Chiu CY, et al. An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. Nucleic Acids Res. 2015;43(7):e46.

28. Ye J, McGinnis S, Madden TL. BLAST: improvements for better sequence analysis. Nucleic Acids Res. 2006;34(Web Server issue):W6-9.

29. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6):1547-9.

30. Le SQ, Gascuel O. An improved general amino acid replacement matrix. Mol Biol Evol. 2008;25(7):1307-20.

31. Zadori Z, Szelei J, Lacoste MC, Li Y, Gariepy S, Raymond P, et al. A viral phospholipase A2 is required for parvovirus infectivity. Dev Cell. 2001;1(2):291-302.

32. Loken T. Border disease in sheep. Vet Clin North Am Food Anim Pract. 1995;11(3):579-95.

33. Thabti F, Fronzaroli L, Dlissi E, Guibert JM, Hammami S, Pepin M, et al. Experimental model of Border Disease Virus infection in lambs: comparative pathogenicity of pestiviruses isolated in France and Tunisia. Vet Res. 2002;33(1):35-45.

34. Dawson M. Pathogenesis of maedi-visna. Vet Rec. 1987;120(19):451-4.

35. Narayan O, Clements JE. Biology and pathogenesis of lentiviruses. J Gen Virol. 1989;70 (Pt 7):1617-39.

36. Benavides J, Fuertes M, Garcia-Pariente C, Ferreras MC, Garcia Marin JF, Perez V. Natural cases of visna in sheep with myelitis as the sole lesion in the central nervous system. J Comp Pathol. 2006;134(2-3):219-30.