Molecular characterisation of equid alphaherpesvirus 1 strains isolated from aborted fetuses in Poland

Anna Karolina Matczuk*, Małgorzata Skarbek, Natalia Anna Jackulak and Barbara Anna Bażanów

Abstract

Background: Equid alphaherpesvirus 1 (EHV-1) is one of the main infectious causative agents of abortion in mares and can also be associated with stillbirth, neonatal foal death, rhinopneumonitis in young horses and a neurological disorder called equine herpesvirus myeloencephalopathy (EHM). The neuropathogenicity of the virus was shown to be significantly higher in EHV-1 strains that carry a single nucleotide point (SNP) mutation in the ORF30, which encodes a catalytic subunit of viral DNA polymerase (ORF30 D752). Another gene, ORF68 is frequently used for phylogenetic analysis of EHV-1.

Methods: 27 EHV-1 strains isolated from aborted equine fetuses in Poland, collected between 1993 and 2017, were subjected to PCR targeting the open reading frames (ORFs) 30 and 68 of the EHV-1 genome. PCR products obtained were sequenced and SNPs were analyzed and compared to sequences available in GenBank.

Results: None of the analyzed sequences belonged to the ORF30 D752 neuropathogenic genotype: all EHV-1 belonged to the non-neuropathogenic variant N752. On the basis of ORF68 sequences, the majority of EHV-1 sequences (76.9%) cannot be assigned to any of the known groups; only six sequences (23.1%) clustered within groups I and IV.

Conclusions: EHV-1 strains obtained from abortion cases belong to the non-neuropathogenic genotype. Many EHV-1 ORF68 sequences have similar SNPs to those already described in Poland, but a clear geographical distribution was not observed. A single particular ORF68 sequence type was observed in strains isolated from 2001 onwards.

Keywords: EHV-1, Abortion in mares, Phylogenetic analysis, ORF30, ORF68, Equine herpesvirus myeloencephalopathy
in the catalytic subunit of the viral DNA polymerase. This single amino acid mutation in the viral polymerase of EHV-1 causes higher tropism to lymphocytes and longer viremia in experimentally infected horses when compared to animals infected with EHV-1 lacking this particular mutation [13].

Although infection with N752 can also cause EHM, infections with D752 increase the risk of developing EHM [3, 6]. For this reason, EHV-1 N752 is referred to as a non-neuropathogenic genotype, and D752 as a neuropathogenic genotype. Other risk factors for EHM, beside the genotype of the virus, include host and environmental factors such as breed, age and sex of the horse [14].

In some neuropathogenic EHV-1 strains, another mutation, a substitution C to A in position 2258 in addition to A to G in position 2254 in the ORF30 gene was observed [15]. Infection with the neuropathogenic genotype of EHV-1 can also lead to severe abortion outbreaks [5]. Analysis of the whole EHV-1 genome sequences of reference neuropathogenic strain Ab4 and the less virulent V592 strain revealed that the highest variation rate occurs in the ORF68 gene, a homologue to the human herpes simplex virus type US2 region [12]. Analysis of 131 field isolates revealed that, indeed, this region of the EHV-1 genome has the highest mutation rate (2%), which allows its nucleotide sequence to be used as a genetic marker to classify virus strains into different groups [12]. The strains were divided into six groups (two strains remained unassigned) on the basis of SNPs in the polymorphic region of ORF68 and the number of G residues within this region (nucleotides 732–739). Therefore, sequencing of ORF68 replaced the restriction fragment length polymorphism in molecular and epidemiological analyses of the EHV-1 strains obtained during outbreaks [16, 17].

The aim of this study was to analyse the neuropathogenic potential and genetic relationship of EHV-1 strains isolated from aborted equine fetuses in Poland.

**Materials and methods**

**Virus strains, cell cultures, DNA extraction**

The 27 field strains analysed in this study were isolated from placentas or internal organs (liver, spleen, lungs) of aborted fetuses. All samples were sent as clinical samples to the Division of Microbiology, Department of Pathology, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences between 1993 and 2017 (Fig. 1, Table 1). The majority of the strains originated from large or medium size breeder studs, and only a few of them from recreational studs. A vaccine strain RacH and DNA from a neuropathogenic strain Ab4 were also included in the study, as positive controls. After the original isolation, the viruses were stored as cell culture supernatants at −80 °C or in liquid nitrogen. Before the isolation of DNA, all strains were cultured on
rabbit kidney cells (RK-13) maintained in Minimum Essential Medium (MEM, Sigma-Aldrich, Germany) at 37 °C in an atmosphere containing 5% CO₂ in T25 flasks until appearance of the cytopathic effect. The flasks were then frozen and thawed and 200 μl of supernatant was subjected to DNA extraction with the QIAmp DNA Mini Kit (Qiagen) according to the manufacturer's instructions, with a final elution volume of 50 μl.

**PCR amplification and sequence analysis**

The amplification of ORF30 was performed with primers described by [13] (ORF30F: 5’-GCTACTTCTGAAAA CGGAGGC-3’; ORF30R5’-CTATCCTCAGACACCGCAA CA-3’). Amplicons of 466 bp were generated with DreamTaq Green Master Mix in a 50 μl reaction volume with 200 nM of forward and reverse primer and 2 μl virus DNA template. The cycling conditions were as follows: initial denaturation at 95 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min and extension at 72 °C for 2 min, followed by one step of final extension at 72 °C for 8 min.

The PCR products were visualized on a 2% agarose gel, bands were excised and purified with the Gel-Out kit (A&A Biotechnology, Poland), then eluted in 50 μl nuclease-free water. The purified products were digested with SalI enzyme (recognition site 5’↓GTCGAC 3’) (EURx, Poland) in a 50 μl reaction volume containing 15 μl of purified PCR product, 5 μl 10x Buffer High, 0.5 μl 100x BSA, and 1 μl SalI enzyme, for 1 h at 37 °C followed by 20 min at 65 °C. The Ab4 and RacH strains were used as positive and negative controls for SalI digestion, respectively. After digestion, the products were

| Serial number of strain | Strain reference number | Date of virus isolation | Virus isolation source | Place of isolation stable/voivodeship | Group |
|-------------------------|-------------------------|-------------------------|------------------------|---------------------------------------|-------|
| 1                       | 93_01                   | 16 March 1993           | fetus                  | A/ZP                                  | II    |
| 2                       | 93_02                   | 8 April 1993            | fetus 10 m             | A/SL                                  | IV    |
| 3                       | 93_03                   | 8 April 1993            | fetus 10 m             | A/SL                                  | PL_2013_V |
| 4                       | 93_04                   | 8 April 1993            | fetus 9 m              | A/SL                                  | partial (PL_2013_V) |
| 5                       | 93_05                   | 8 April 1993            | fetus 6 m              | B/WP                                  | II    |
| 6                       | 95_01                   | 23 January 1995         | fetus                  | A/WP                                  | PL_2013_V |
| 7                       | 95_02                   | 28 January 1995         | fetus                  | A/WP                                  | II    |
| 8                       | 96_01                   | 12 February 1996        | fetus 8 m              | A/WP                                  | PL_2013_V |
| 9                       | 97_01                   | 15 January 1997         | fetus 8 m              | A/LB                                  | PL_1999_II |
| 10                      | 97_02                   | 23 February 1997        | fetus 10 m             | A/LB                                  | PL_1999_II |
| 11                      | 97_03                   | 23 February 1997        | fetus 8-9 m            | A/LB                                  | PL_1999_II |
| 12                      | 97_04                   | 25 February 1997        | fetus 10 m             | A/LB                                  | partial (PL_2001_I) |
| 13                      | 97_05                   | 25 February 1997        | fetus                  | A/LB                                  | partial (PL_2001_I) |
| 14                      | 97_06                   | 23 March 1997           | fetus 10 m             | A/LB                                  | partial (PL_2001_I) |
| 15                      | 99_01                   | 20 February 1999        | fetus 4 m              | A/SL                                  | partial/NA |
| 16                      | 99_02                   | 22 February 1999        | fetus 10 m             | A/DS                                  | partial (PL_2001_I) |
| 17                      | 99_03                   | 22 February 1999        | fetus 10 m             | A/DS                                  | partial (PL_2001_I) |
| 18                      | 00_01                   | 10 February 2000        | fetus 11 m             | B/WP                                  | II    |
| 19                      | 00_02                   | 27 February 2000        | fetus 9 m              | A/LS                                  | II    |
| 20                      | 01_01                   | 28 May 2001             | fetus                  | B/DS                                  | PL_1999_II |
| 21                      | 01_02                   | 29 October 2001         | fetus 7 m              | A/DS                                  | Not tested |
| 22                      | 04_01                   | 17 November 2004        | fetus 8 m              | B/ZP                                  | PL_1999_II |
| 23                      | 05_01                   | 21 January 2005         | fetus 7 m              | A/LD                                  | PL_1999_II |
| 24                      | 06_01                   | 16 February 2006        | fetus 9 m              | B/SL                                  | PL_1999_II |
| 25                      | 10_01                   | 23 February 2010        | fetus                  | A/MZ                                  | PL_1999_II |
| 26                      | 10_02                   | 25 February 2010        | fetus                  | A/MZ                                  | PL_1999_II |
| 27                      | 17_01                   | 5 March 2017            | fetus                  | C/DS                                  | PL_1999_II |

Characteristic of isolated strains. M – months. Groups: II and IV [12], PL_1999_II and PL_2013_V [19]. Partial means that the sequence is not complete, but sequence has SNPs the same as indicated in brackets. Abbreviations of the voivodeships are explained in Fig. 1.
visualized by electrophoresis on 2% agarose gel. The remaining 35 μl of purified PCR products were subjected to sequencing with ORF30F primer (Genomed, Poland).

Amplification of the 645 bp region of ORF68 was performed with primers described by Nugent et al. [12] (ORF68F: 5’CAAGAAACCTGCTCAACC3’; ORF68R: 5’AGCATTCACCACGTTCC3’). The PCR was performed with Agilent’s Herculese II fusion DNA polymerase (Agilent Technologies, Santa Clara, USA), with the same conditions as described by Negussie et al. [10]. The PCR products were visualized on 2% agarose gels, the bands were excised, purified with the Gel-Out kit or by a sequencing company (A&A Biotechnology, Poland), and subjected to sequencing with at least two primer sets: ORF68F primer and for some products with ORF68R or ORFS1 primer 5’GAAGATAGAATGGGTGTGAG3’ (Genomed, Warsaw Poland; GATC Cologne, Germany). Chromatograms obtained from sequencing were manually checked for errors with FinchTV software. The fragments of ORF68 sequences obtained were aligned with a ClustalW algorithm, with a set of representative sequences of each group from the original Nugent et al. 2006 study [12] and a recent Polish study Stasiak et al. [18] obtained from GenBank with MEGA7.1 software [19]. The ORF68 sequences obtained in this study were subjected to GenBank under the accession numbers MH329902-H329927.

Results

PCR products with a size of approximately 450 bp from 27 Polish EHV-1 strains were obtained from the ORF30 region of the virus genome. The SalI digestion of PCR products was negative for EHV-1 samples (Fig. 2). The sequencing of the PCR products further confirmed that all analysed sequences had adenine in position 2254. Guanidine was not observed in ORF30, meaning that all 27 strains isolated from aborted equine fetuses in Poland between 1993 and 2017 belonged to the non-neuropathogenic variant (N752) of EHV-1. No other mutations, including substitution C to A in position 2258, were observed in analyzed ORF30 sequences. Sequences of EHV-1 ORF30 are added in Additional file 1.

PCR products with a size of approximately 900 bp were generated from the ORF68 region of the genome including approximately 600-bp-long polymorphic segments. Products were sequenced and aligned to identify SNPs. These SNPs are presented in Fig. 3. The nucleotide sequence of the Ab4 strain (GB80_1_1) as a member of EHV-1 Group 1 served as a basis for the comparison of nucleotide changes. The sequences obtained from sample 01_02 had lower quality despite multiple sequencing efforts; therefore this strain was excluded from the analysis. The sequences obtained for 93_04, 97_05, 97_06, 99_01 and 99_03 are shorter than the others, due to poorer sequencing coverage for region nt 720–760, but were included in the analysis. Out of 26 Polish EHV-1 sequences analysed in this study, five (19.2%) belonged to group II and one (3.8%) belonged to group IV, while the remaining 20 (76.9%) were not classified within any of the groups originally described by Nugent et al. [12] (Table 1 and Fig. 3). More than half of the EHV-1 sequences (57.7%) contained A629 SNP. Ten Polish EHV-1 sequences (38.5%) possessed the same substitutions (A629 and T750) as the EHV-1 sequence of GB86_3_2, which was described as an unassigned sequence in the original study.

![Fig. 2 Picture of the SalI digestion of the PCR product of ORF30. M: molecular marker Gene Ruler Plus (Thermo), size shown in bps. 1–27 ORF30 PCR products digested with SalI. The numbers 1–27 correspond to the numbers of strains listed in Table 1. Ab4: neuropathogenic strain, RacH: vaccine strain, non-neuropathogenic.](image-url)
by Nugent et al. [12] and Polish EHV-1 sequence PL_1999_II from a recent study by Stasiak et al. [18]. Three sequences had the same SNPs (A_629, C_626 and T_750) as sequence PL_2013_V, while two sequences were similar to PL_2001_I from the Stasiak et al. [18] (Figs. 2 and 3).

All of the analysed ORF68 sequences possessed seven G residues in a homopolymeric tract compared to eight G residues in the Ab4 strain (nucleotides 732–739). The number of G residues was not established for 93_04, 97_05, 97_06, 99_01 and 99_03 due to the shorter sequences available for those strains.

A clear geographical distribution is difficult to establish (Table 1) since group “PL_1999_II” sequences were found in 7 different voievodenships in contrast to the group II described by Nugent et al. [12] which was found only in western voievodenships. Also, some sequences isolated from the same stud within a similar time period differed, e.g., in the 1997 Lubelskie voievodenship (LB) outbreak isolates 97_01–97_03 contained a A_629 SNP, vs. a T_562 SNP of isolates 97_04–97_06. Two EHV-1 sequences isolated in the same stud over several years (B/WP-Table 1) clustered within group II, which may indicate that a similar virus was present in that region for 7 years.

Discussion

The strains included in this study originated from abortion cases that occurred between October and May, which is the breeding season in mares (Table 1) [4].

In this study, none of the EHV-1 strains isolated from aborted fetuses between 1993 and 2017 belonged to the neuropathogenic genotype D752, and all belonged to the non-neuropathogenic variant N752. In two recent studies conducted in Poland, the neuropathogenic variant D752 was found in 2 out of 20 cases (10%) and in none out of 37 (0%) EHV-1 isolates obtained from abortion cases [18, 20]. In those studies, Polish D752 EHV-1 strains were isolated in the years 2009 and 2010. Our study includes older isolates than previous Polish studies done by Stasiak et al. [18, 20]. Summarizing all the analyses so far, on Polish EHV-1 sequences from years 1993 to 2017, the prevalence of the neuropathogenic genotype D752 is very low, only 3.07% (2/65 EHV-1). In all three studies (this one and [18, 20]), the abortions were not associated with neurological symptoms in the horse premises. It seems that non-neuropathogenic EHV-1 strains are prevalent in Poland. To the author’s knowledge, EMH was never described in Poland, although horse practitioners report isolated incidents of neurological symptoms in horses, that are, however, not supported by any laboratory diagnosis of EHV-1. This is in contrast to other countries, where devastating outbreaks of EHM were reported [9, 10, 21, 22].

While studies indicate that the non-neuropathogenic N752 (nt A2254) variants are more common, the prevalence of D752 strains have increased in recent decades in the USA and some European countries [6, 15]. However, a similar increase has not been observed in other
countries such as Japan or New Zealand [23, 24]. Recent studies suggest that neuropathogenic strains could have a selective advantage over non-neuropathogenic strains which have increased their prevalence in horse populations [25]. A very low prevalence of D752 strains and an absence of EHM outbreaks in Poland could be due to the late introduction of these strains in Poland, therefore it is recommended to monitor the genotype of EHV-1 in the future. If the D752 strains were more prevalent, this could influence quarantine rules and infectious disease management in studs. It could also influence the amount of samples sent for diagnostics.

The use of the ORF68 sequence as a molecular marker associated with the geographical origin of EHV-1 was first proposed by Nugent et al. [12]. However, more data now available for this sequence indicate that many sequences cannot be classified within already established groups. In a similar study performed in Hungary, 65.7% of EHV-1 isolates were grouped according to the classification of Nugent et al. [12] into groups II, III and IV, while the remaining isolates formed four separate groups [17]. In a recent Polish study, the main EHV-1 groups were also III and IV, while the majority of sequences were matched either by the Nugent “unassigned” group or formed a separate group [18]. In our study, analysis of the geographical distribution of strains isolated in different regions in Poland have not revealed any pattern or clustering. In fact, similar sequences have been isolated from multiple regions, and the others appear only in one stud in a particular year. These findings are in agreement with previous studies conducted in Hungary and Poland [17, 18]. In this study, we observed different EHV-1 strains that caused abortions in the same stud within a similar time period. This could suggest that at least some of these abortions might have been caused by reactivation of a persistent EHV-1 infection rather than a single introduction of a new virus into a stud’s premises (stable A/LB, Table 1). It is proposed that EMH and abortions can be caused by virus shed during the reactivation of latency and transmission to susceptible horses in the stud [3, 26]. All the recent isolates of EHV-1 sequenced in this study belong to group PL_1999 II. This particular ORF68 sequence appeared in 1997 and is the only sequence isolated since 2001, suggesting that these EHV-1 strains possess advantages in transmission or more often induce abortion in mares.

Conclusions
In summary, equine abortion cases that occurred in Poland between 1993 and 2017 were caused by EHV-1 with a non-neuropathogenic ORF30 N752 genotype. On the basis of ORF68 sequences, the majority of EHV-1 strains cluster within groups II, IV or cannot be assigned to any of the known groups, but show similarity to those already described in Poland.

Additional file

**Additional file 1: Nucleotide sequences obtained from sequencing of ORF30 PCR products.** Data is presented in FASTA format. (FAS 8 kb)

**Abbreviations**
EHV-1 equine herpesvirus 1; EHV-4 equine herpesvirus myeloencephalopathy; n.t.: nucleotide; ORF: Open reading frame; SNP: Single point mutation

**Acknowledgments**
We thank professor Jerzy Rola and Karol Stasiak from the Department of Virology, National Veterinary Research Institute, Pulawy, Poland, for the DNA sample of neuropathogenic EHV-1 Aab4 strain.

**Funding**
Publication was supported by Wroclaw Centre of Biotechnology, Programme The Leading National Research Centre (KNOW) for years 2014–2018.

**Availability of data and materials**
The sequences generated and analysed during the current study are available in the Genbank repository.

**Authors’ contributions**
AKM and BAB conceived and designed the experiments; AKM, MS, and NAJ performed the experiments; AKM analysed sequencing data; AKM wrote the manuscript; BAB revised the manuscript. All authors read and approved the final manuscript.

**Ethics approval**
Not applicable - the work was conducted on clinical samples sent by veterinary practitioners.

**Consent for publication**
Not applicable

**Competing interests**
The authors have declared no competing interests.

**Publisher’s Note**
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Received:** 25 May 2018 **Accepted:** 9 November 2018 **Published online:** 03 December 2018

**References**
1. Bazanow BA, Fracka AB, Jackulak NA, Staroniewicz ZM, Ploch SM. A 34-year retrospective study of equine viral abortion in Poland. Pol J Vet Sci. 2014;17:607–12.
2. Allen GP, Kydd JH, Slater D, Smith KC. Equid herpesvirus 1 and equid herpesvirus 4 infections. In: Coetzer JAW, Tusin RC, editors. Infectious diseases of livestock. 1st ed. Newmarket: Oxford University press; 2004. p. 829–59.
3. Lunn DP, Davis-Poynter N, Flaminio MBF, Horohov DW, Osterrieder K, Pusterla N, Townsend HGG. Equine herpesvirus-1 consensus statement. J Vet Intern Med. 2009;23:450–61.
4. Bazanow BA, Jackulak NA, Fracka AB, Staroniewicz ZM. Abortogenic viruses in horses. Equine Vet Educ. 2014;26:48–55.
5. Damiani AM, de Vries M, Reimers G, Winkler S, Osterrieder N, Severe Equine A. Herpesvirus type 1 (EHV-1) abortion outbreak caused by a Neuropathogenic strain at a breeding farm in northern Germany. Vet Microbiol. 2014;172:555–62.
6. Perkins GA, Goodman LB, Tsujimura K, Van de Walle GR, Kim SG, Dubovik EJ, Osterrieder N. Investigation of the prevalence of neurologic equine herpes virus type 1 (EHV-1) in a 23-year retrospective analysis (1984–2007). Vet Microbiol. 2009;139:375–8.
7. Visani MA, Becerra ML, Olguin Perghone C, Tordoya MS, Mino S, Barrandeguy M. Neuropathogenic and non-neuropathogenic genotypes of equid herpesvirus type-1 in Argentina. Vet Microbiol. 2009;139:361–4.
8. Fritsche AK, Borchers K. Detection of neuropathogenic strains of equid herpesvirus – 1 (EHV-1) associated with abortions in Germany. Vet Microbiol. 2010;147:176–80.

9. Pronost S, Legrand I, Ptel PH, Wegge B, Lissens J, Freymuth F, Richard E, Fortier G. Outbreak of equine herpesvirus Myeloencephalopathy in France: a clinical and molecular investigation. Transbound Emerg Dis. 2012;59:256–63.

10. Negusse H, Gizaw D, Tessema TS, Nauwynck HJ. Equine Herpesvirus-1 Myeloencephalopathy, an emerging threat of working equids in Ethiopia. Transbound Emerg Dis. 2015;62:389–39.

11. Telford EAR, Watson MS, McBride K, Davison AJ. The DNA sequence of equine Herpesvirus-1. Virology. 1992;189:304–16.

12. Nugent J, Birch-Machin I, Smith KC, Mumford JA, Swann Z, Newton JR, Bowden RJ, Allen GP, Davis-Poynter N. Analysis of equid herpesvirus 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with Neuropathogenic versus Nonneuropathogenic disease outbreaks. J Virol. 2006;80:4047–60.

13. Goodman LB, Loregian A, Perkins GA, Nugent J, Buckles EL, Mercorelli B, Kydd IH, Palu G, Smith KC, Osterrieder N, Davis-Poynter N. A point mutation in a herpesvirus polymerase determines Neuropathogenicity. PLoS Pathog. 2007;3:e160.

14. Goehring LS, van Winden SC, van Maanen C, Siet van Oldruitenborgh-Oosterbaan MW. Equine Herpesvirus Type 1-Associated Myeloencephalopathy in The Netherlands: A Four-Year Retrospective Study (1999–2003). J Vet Intern Med. 2010;20:601–7.

15. Smith KL, Allen GP, Branscum AJ, Cook RF, Vickers ML, Timoney PJ, Balasuurya UB. The increased prevalence of Neuropathogenic strains of EHV-1 in equine abortions. Vet Microbiol. 2010;141:5–11.

16. Palfi V, Christensen LS. Analyses of restriction fragment patterns (RFPs) and pathogenicity in baby mice of equine herpesvirus 1 and 4 (EHV-1 and EHV-4) strains circulating in Danish horses. Vet Microbiol. 1995;47:199–204.

17. Malik P, Bálint A, Dán A, Pálfi V. Molecular characterisation of the ORF68 region of equine Herpesvirus-1 strains isolated from aborted fetuses in Hungary between 1977 and 2008. Acta Vet Hung. 2012;60:175–87.

18. Stasiak K, Dunowska M, Hills SF, Rola J. Genetic characterization of equid herpesvirus type 1 from cases of abortion in Poland. Arch Virol. 2017;162:2329–35.

19. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4.

20. Stasiak K, Rola J, Pliszczys, G. Socha W, Zmudzinski JF. Detection of the Neuropathogenic variant of equine herpesvirus 1 associated with abortions in mares in Poland. BMC Vet Res. 2015;11:102.

21. Walter J, Seeh C, Fey K, Bleul U, Osterrieder N. Clinical observations and Management of a Severe Equine Herpesvirus Type 1 outbreak with abortion and encephalomyelitis. Acta Vet Scand. 2013;55:19.

22. McFadden AMJ, Hanlon D, McKenzie RK, Gibson I, Bueno IM, Pullford DJ, Orr D, Dunowska M, Stanislawek WL, Spence RP, McDonald WL, Munro G, Mayhew IG. The first reported outbreak of equine herpesvirus Myeloencephalopathy in New Zealand. N Z Vet J. 2016;64:125–34.

23. Tsujimura K, Oyama T, Katayama Y, Muranaka M, Bannai H, Nemoto M, Yamazaki T, Kondo T, Kato M, Matsumura T. Prevalence of equine herpesvirus type 1 strains of Neuropathogenic genotype in a major breeding area of Japan. J Vet Med Sci. 2011;73:1663–7.

24. Dunowska M, Gopakumar G, Perrott MR, Kendall AT, Waropasratrakul S, Hartley CA, Carlsake HB. Virological and serological investigation of equid herpesvirus 1 infection in New Zealand. Vet Microbiol. 2015;176:219–28.

25. Franz M, Goodman LB, Van de Walle GR, Osterrieder N, Greenwood AD. A Point Mutation in a Herpesvirus Co-Determines Neuropathogenicity and Viral Sheding. Viruses. 2017;9:6.

26. Slater JD, Borchers K, Trackay AM, Field HJ. The trigeminal ganglion is a location for equine herpesvirus 1 latency and reactivation in the horse. J Gen Virol. 1994;75:2007–16.