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Genetic Variation in the Glycoprotein B Sequence of Equid Herpesvirus 5 among Horses of Various Breeds at Polish National Studs

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Abstract: Equid herpesvirus 5 (EHV-5) is one of two -herpesviruses that commonly infect horses worldwide. The objective of the study was to estimate the genetic variability within EHV-5 viruses circulating among horses in Poland. Partial glycoprotein B (gB) sequences from 92 Polish horses from 13 studs throughout Poland were compared to each other and to three EHV-5 sequences from other countries. Despite the overall high level of conservation, considerable variability was observed around the putative furin cleavage site. Based on phylogenetic analysis, the viruses clustered within two major lineages (A and B), with further sub-clustering within group A. The clustering of EHV-5 sequences was independent of age or geographical origin of the sampled horses. Recombination was identi ed as one of the factors contributing to the genomic heterogeneity. Viruses from unweaned foals were more similar to viruses from other foals at the same stud than to viruses form their dams, suggesting the horizontal transfer and/or evolution of EHV-5 within individual hosts. Our data indicate that the gB sequence is not suitable for tracking the source of EHV-5 infection. Further research is needed to elucidate the importance of the sequence variability around the EHV-5 gB furin cleavage site on the biology of the virus.

Keywords: equid herpesvirus 5; genetic variability; glycoprotein B; phylogeny

Citation: Stasiak, K.; Dunowska, M.; Trewick, S.; Rola, J. Genetic Variation in the Glycoprotein B Sequence of Equid Herpesvirus 5 among Horses of Various Breeds at Polish National Studs. Pathogens 2021, 10, 322. https://doi.org/10.3390/pathogens10030322

Academic Editor: Jochen Bodem

Received: 2 February 2021
Accepted: 6 March 2021
Published: 9 March 2021

1. Introduction

Horses are natural hosts to ve herpesviruses, including equid herpesvirus (EHV) type 5 [1]. This virus is classi ed in the family Herpesviridae, subfamily Gammaherpesvirinae, and genus Percavirus [2]. It shares sequence similarity with another equine gammaherpesvirus EHV-2, as well as with human herpesvirus 4 (Epstein–Barr virus, EBV) [3]. It has been detected from horses worldwide with the higher frequency of detection in young horses compared to older ones [4–6]. Following primary infection, which often occurs early in life, the virus establishes lifelong latent infection in B lymphocytes [7].

The pathogenic potential of EHV-5 has not been fully elucidated, as the virus has been detected from healthy horses as well as from horses with varied clinical presentations including poor performance syndrome, mild respiratory disease, pneumonia, and ocular disease [1,8,9]. The most serious, albeit rare, disease that has been linked to EHV-5 infection in mature horses is equine multinodular pulmonary brosis [10,11].

Very limited genomic data are currently available for EHV-5, with only one fully sequenced genome of the Australian prototype isolate EHV-5 2-141/67 deposited in GenBank [12]. The linear, double-stranded DNA genome of EHV-5 is 179 kbp long and consists of the 79 functional open reading frames (ORFs) with a mean G + C content of 52% [12,13]. Typically for herpesviruses, family-conserved genes are interspersed with species-speci c genes and non-coding regions [3,12]. The conserved herpesviral genes include a gene

coding for glycoprotein B (gB). Glycoprotein B is a highly conserved glycoprotein with homologues among all members of the family Herpesviridae [14]. It plays a pivotal role in the herpesvirus life cycle, including virus entry and cell-to-cell spread [15]. The gB of both EHV-2 and EHV-5 is a disulfate-linked heterodimer that forms an integral part of the viral envelope [16,17]. Glycoprotein B has been commonly used in phylogenetic analysis of herpesviral genomes [18].

Previously, we have reported the high prevalence of equid -herpesvirus infections among Polish horses of various breeds and ages [19]. As part of that study, we also reported considerable heterogeneity of Polish EHV-2 sequences, which was in agreement with similar data from other countries [20,21]. Limited data are available for EHV-5 genomic variability, but results from some studies suggest that the genomic heterogeneity of EHV-5 is lower than that observed for EHV-2 [22,23]. However, others showed high genomic heterogeneity of the analyzed EHV-5 sequences [24–27]. Only two studies investigated the changes in EHV-5 genotypes obtained from individual horses over time [27,28]. Both showed that genotypes varied between individual horses and that more than one genotype could be occasionally recovered from the same nasal swab sample. The most recent and the largest of the studies investigating EHV-5 genomic heterogeneity involved phylogenetic analysis of approximately 460 bp of gB from 34 EHV-5 sequences [28]. Based on this analysis, the authors proposed the existence of four main genotype groups labeled I to IV, with <2% variability at the nucleotide level within each group.

Figure 1. Alignment of deduced partial glycoprotein B sequence from equid herpesvirus 5 (EHV-5) from Arabian horses in stud I including three mare/foal pairs (horses 1/2, 3/4, and 5/6). Two EHV-5 sequences from Iceland and one from Australia are included for comparison. Residues that differ from those in the reference Australian sequence (strain 2-141/67) are highlighted. The putative furin cleavage site is marked with a red box. Gray boxes indicate putative N-glycosylation sites and arrows point to conserved cysteine residues.

Figure 2. Amino acid sequence logo around the putative furin cleavage site, as indicated by a blue arrow (aa 422–426 in AF050671). The region included in the logo spans aa 226 to 358 in AF050671. The logo was generated from the alignment of 95 partial EHV-5 glycoprotein B sequences including 92 sequences from the current study and three international sequences (accession numbers AF050671, GQ325592, and GQ325593). The graph underneath the logo shows the mean pairwise identity of all pairs in the columns (green: 100%, brown: more than 30% but less than 100%, red: less than 30%).

2.2. Phylogeny

Based on the phylogenetic tree constructed from alignment of the full available sequence (1.2 kbp) of EHV-5 gB (Figure S1), the 95 EHV-5 sequences clustered within two main lineages. Most (n = 58) were clustered within a large group designated group A that could be further divided into at least three sub-clusters containing 42 (cluster A1), 3 (cluster A2), and 13 (cluster A3) EHV-5 sequences.

Cluster A3 included EHV-5 isolate 281 from Iceland. The second group (B) contained 37 EHV-5 sequences including two overseas EHV-5 sequences (Australian 2-141/67 and Icelandic BB5-5). Based on the phylogenetic tree inferred from the alignment of a shortened fragment of EHV-5 that included an additional 27 EHV-5 Swedish and three British EHV-5 sequences previously analyzed by Back and others [28], sequences from groups designated

Sequences from cluster A2 were identi ed as putative recombinants and hence were not included in the network analyses. The haplotype network structure did not correlate with horse age group or with the geographical location of the horses sampled, which was supported by the results of AMOVA (Table 1).

Table 1.

It was clear from the phylogenetic analysis that several different EHV-5 genotypes circulated among horses at several studs. Sequences from some studs (e.g., Stud I or III) were distributed across the entire tree, within all four main lineages. Sequences from other studs appeared less variable. For example, all five sequences from Stud XII and 6/7 sequences from Stud IV clustered within group B (Figure S1). As horses from different studs belonged to different breeds, this may suggest the existence of some breed-related differences. However, it is equally possible that such differences represent differences in the number of viral genotypes circulating among horses or the number and management of horses sampled at each stud.

Even within studs with highly variable EHV-5 sequences, some horses were infected with viruses that were identical to each other over the length of the analyzed gB fragment suggesting a horizontal spread. This can be exemplified by EHV-5 sequences from horses 17, 18, and 20 at Stud I, sequences 83, 86, 87, and 88 at Stud XI, or sequences 89 and 92 at Stud XII. However, sequences from horses 26 (Stud II) and 38 (Stud IV) were also 100% identical to each other, even though they originated from horses from different variable them h

recombination has been shown to contribute to the genomic variability observed among other herpesviruses [32–34]. However, the removal of putative recombinant sequences from the analyses did not reduce the level of variability observed between the remaining EHV-5 sequences, suggesting that mechanisms other than recombination must have contributed to the evolution of those sequences.

It has been shown previously that EHV-5 gB is incorporated into the viral envelope in the cleaved form, with the sizes of the cleaved fragments consistent with furin cleavage [16]. All 92 predicted gB sequences from Polish EHV-5 had a conserved putative furin cleavage site, which was consistent with reports by others using smaller datasets [16,28]. Although the canonical motif for furin cleavage has been described as R-X-[K/R]-R #, several variations to this motif have been recognized, including R-X-R-K, which is present in at least one of the Polish sequences [35]. Furin cleavage plays a role in a variety of biological processes including the facilitation of infection by viruses from several families including Herpesviridae [36], Coronaviridae [37], Papillomaviridae [38], or Pneumoviridae [39]. The region around the furin cleavage site has been shown to be under positive selection during infection with human cytomegalovirus [40] despite the fact that proteolytic processing by furin is not essential for HCMV growth in cell culture [41]. The conservation of this site among all 95 EHV-5 sequences included in the analysis suggest that furin cleavage is important for EHV-5 gB processing during EHV-5 infection, but the exact effects of furin cleavage on EHV-5 life cycle remain to be determined.

Ten cysteine residues that form five disulfide linkages bonds important for the three-dimensional structure of herpesviral gB are conserved among gB homologs, five of which are located within the fragment analyzed in the current study [14,16,42]. All five were conserved between all 95 EHV-5 sequences analyzed (aa positions 52, 117, 201, 249 and 379 in Figure 1). Holloway and others [16] also reported that EHV-5 gB was N-glycosylated and contained 16 putative N-glycosylation sites, three of which were conserved between sequences of nine γ-herpesviruses analyzed in that study (aa 170, 554, and 630 in AF050671). One of those predicted N-glycosylation sites (aa 170, corresponding to position 74 in Figure 1) was located within the fragment analyzed in the current study and was conserved between all 95 sequences. Several other putative N-glycosylation sites were conserved between all EHV-5 sequences in the current study, with some variability between the exact position of N-glycosylation sites around the putative furin cleavage site, highlighting the importance of N-glycosylation for the processing and function of herpesviral gB [16,17,43].

The methodology employed in the current study did not allow for detection of multiple genotypes from the same sample, as PCR products were sequenced directly, without prior cloning or using next-generation sequencing. The presence of multiple genotypes in the sample may be one possible explanation for the number of unresolved sites in sequences from eight horses that were excluded from the analysis. Another limitation of the sampling strategy is a lack of multiple samples from individual horses over time.

In summary, we have presented a comprehensive analysis of a large set of partial EHV-5 gB sequences. Our data further supported the presence of conserved features in EHV-5 gB sequences. However, there was also a considerable variation between EHV-5 sequences from individual horses, even among horses that have been in close contact with each other. The sequence variability was concentrated around the putative furin cleavage site, and it was not correlated with geographical origin or the age of sampled horses. The implication of the changes within this region on interactions between the virus and its host need further elucidation.

4. Materials and Methods

4.1. Sample Collection

DNA collected from nasal swab samples obtained as part of a separate study [19] were used as a starting material. Brie y, horses (n

Figure 4. Map of Poland showing the location of the studs (denoted by Arabic numbers) and the number of EHV-5 positive horses from each stud that were included in the current study.

At each stud, horses of different ages including foals, mares, yearlings and 2-year-olds were included. Horses from Studs II and III showed clinical signs of respiratory disease at the time of sampling. The remaining horses were healthy at the time of sample collection. The collection and processing of samples have been described in detail elsewhere [19]. The swabs were tested for the presence of EHV-1, EHV-4, EHV-2, and EHV-5. Nearly half (254/540, 47.0%) of the sampled horses tested positive for EHV-5, either alone or in combination with other EHVs [19

were subjected to electrophoresis through a 1.5% ethidium bromide stained agarose gel, puri ed (ExoSAP-IT PCR Product Cleanup Reagent, Thermo Fisher Scienti c, Santa Clara, CA, USA), and sequenced in both directions with the same primers used for ampli cation using BigDye® Terminator version 3.1 (Applied Biosystems, Austin, TX, USA) on a 3730 xl DNA Analyzer (Applied Biosystems) at the Genomed (Warsaw, Poland).

4.3. Sequence Analyses

The obtained sequences were assembled using Geneious 9.1.8 (https://www.geneious.com, accessed on 8 March 2021) and trimmed to exclude low-quality ends. The 5′ and 3′ ends of all trimmed sequences corresponded to nucleotides 404 and 1621, respectively in the sequence of the reference Australian strain EHV-5. 2-141/67 (GenBank accession AF050671). The nucleotide sequences were translation-aligned using MUSCLE amino acid alignment within Geneious v9.1.8 (Biomatters, Ltd., Auckland, New Zealand) [44]. Both full alignments of 95 sequences and an alignment of subset sequences from Stud I were generated. A phylogenetic tree was inferred using the maximum likelihood method with 500 bootstrap replicates using the K2+G+I substitution model in MEGAX (version 10.05) software [45]. An additional tree was constructed using an alignment of a shorter fragment of gB (450 nucleotides) in order to include Swedish (n = 27) and British (n = 3) EHV-5

Funding: This research received no external funding.

Institutional Review Board Statement: An informed approval was sought from stud managers before commencement of sampling. One of the roles of the National Veterinary Research Institute in Pulawy is monitoring of endemic diseases among Polish livestock. The sampling for the current study was performed within the scope de ned by this role. The approval from ethics committee was not required according to national regulation ("Act on the Protection of Animals Used for Scienti c or Educational Purposes" published in the Journal of Laws of 2015, item 266 from 15 January 2015).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data sets supporting the results of this article are included within the article. The nucleotide sequences of Polish EHV-5 described in this study were submitted to GenBank under the accession numbers: MW526273—MW526364.

Acknowledgments: We wish to thank the managers of the studs and eld veterinarians for their help with sample collection and Mary Morgan-Richards for her helpful comments and suggestions.

21. Brault, S.A.; Bird, B.H.; Balasuriya, U.B.; MacLachlan, N.J. Genetic heterogeneity and variation in viral load during equid herpesvirus-2 infection of foals. Vet. Microbiol. 2011, 147, 253–261. [CrossRef]
22. Bell, S.A.; Balasuriya, U.B.; Gardner, I.A.; Barry, P.A.; Wilson, W.D.; Ferraro, G.L.; MacLachlan, N.J. Temporal detection of equine herpesvirus infections of a cohort of mares and their foals. Vet. Microbiol. 2006, 116, 249–257. [CrossRef]
23. Lee, S.K.; Lee, I. The Molecular Detection of Equine Herpesviruses 2 and 5 in Genital Swabs From Clinically Normal Thoroughbred Mares in South Korea. J. Equine Vet. Sci. 2019, 79, 68–72. [CrossRef]
24. Negussie, H.; Gizaw, D.; Tesfaw, L.; Li, Y.; Oguma, K.; Sentsui, H.; Tessema, T.S.; Nauwynck, H.J. Detection of Equine Herpesvirus (EHV) -1, -2, -4 and -5 in Ethiopian Equids with and without Respiratory Problems and Genetic Characterization of EHV-2 and EHV-5 Strains. Transbound Emerg. Dis. 2017, 64, 1970–1978. [CrossRef] [PubMed]
25. Ataseven, V.S.; Bilge-Dagalp, S.; Oguzoglu, T.C.; Karapinar, Z.; Guzel, M.; Tan, M.T. Detection and sequence analysis of equine gammaherpesviruses from horses with respiratory tract disease in Turkey. Transbound Emerg. Dis. 2010, 57, 271–276. [CrossRef]
26. Radalj, A.; Nišavić, J.; Krnjaić, D.; Valcić, M.; Jovanović, T.; Veljović, L.; Milić, N. Detection and molecular characterization of equine herpesviruses 1, 2, and 5 in horses in the Republic of Serbia. Acta Vet. Brno 2018, 87, 27–34. [CrossRef]
27. Dunowska, M.; Holloway, S.A.; Wilks, C.R.; Meers, J. Genomic variability of equine herpesvirus-5. Arch. Virol. 2000, 145, 1359–1371. [CrossRef] [PubMed]
28. Back, H.; Ullman, K.; Leijon, M.; Soderlund, R.; Penell, J.; Stahl, K.; Pringle, J.; Valarcher, J.F. Genetic variation and dynamics of infections of equid herpesvirus 5 in individual horses. J. Gen. Virol. 2016, 97, 169–178. [CrossRef]
29. Torfason, E.G.; Thorsteinsdottir, L.; Torsteinsdottir, S.; Svansson, V. Study of equid herpesviruses 2 and 5 in Iceland with a type-speci c polymerase chain reaction. Res. Vet. Sci. 2008, 85, 605–611. [CrossRef] [PubMed]