




# Subclinical infection and potential shedding routes of equine parvovirus-hepatitis among hospitalized horses in Austria

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## Abstract

**Background:** Equine parvovirus hepatitis (EqPV-H) can cause Theiler's disease and subclinical hepatitis in horses.

**Objectives:** Assess the frequency of subclinical EqPV-H infection in hospitalized horses and to study viral transmission by investigating potential shedding routes.

**Animals:** One hundred sixteen equids, that presented to the University Equine Hospital of the University of Veterinary Medicine Vienna between February 2021 and March 2022, for causes other than hepatopathy.

**Methods:** In this cross-sectional study, samples (serum, feces, nasal, and buccal swabs) of hospitalized horses were collected. Sera were screened for the presence of anti-EqPV-H antibodies by a luciferase immunoprecipitation system assay. Quantitative PCR was used for the detection of EqPV-H DNA in the samples and a nested PCR was used for further validation.

**Results:** Seroprevalence was 10.3% (12/116) and viremia occurred in 12.9% (15/116) of the serologically positive horses. The detected viral load in serum varied from non-quantifiable amount to  $1.3 \times 10^6$  genome equivalents per milliliter of serum. A low viral load of EqPV-H DNA was detected in 2 nasal swabs and 1 fecal sample.

**Conclusion and Clinical Importance:** EqPV-H DNA was detected in nasal secretions and feces of viremic horses, which could pose a risk to naive hospitalized horses. It is advisable to screen hospitalized horses that are potential donors of blood or plasma to reduce the risk of iatrogenic EqPV-H transmission.

## KEYWORDS

hepatic viruses, horizontal transmission, horse, hospital hygiene, prevalence, Theiler's disease

**Abbreviations:** DNA, deoxyribonucleic Acid; EqPV-H, equine parvovirus hepatitis; GE, genome equivalents; HPVB19, human parvovirus B19; LIPS, luciferase immunoprecipitation system; nPCR, nested polymerase chain reaction; NCBI, National Center for Biotechnology Information; NS1, non-structural protein 1 (of EqPV-H); PCR, polymerase chain reaction; q-PCR, quantitative polymerase chain reaction; RLU, relative light units; TTC17, tetratricopeptide repeat domain 17.

## 1 | INTRODUCTION

Equine parvovirus hepatitis (EqPV-H) is a single-stranded, non-enveloped, hepatotropic DNA virus belonging to the family of

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*Parvoviridae*.<sup>1,2</sup> There is a worldwide distribution of EqPV-H with 3.2%–19.8% DNA prevalence in serum and 15%–34.6% antibody seroprevalence.<sup>1,3–13</sup> Horses infected with EqPV-H have various clinical presentations.<sup>14</sup> Infected horses might develop acute hepatic necrosis, which is known as Theiler's Disease, a life-threatening hepatopathy.<sup>1,14,15</sup> However, in many cases the infection might lead to a self-limiting condition, in which horses maintain a clinically normal state without elevated serum activity of liver enzymes, or have a mild elevation of serum activity of liver enzymes with or without clinical signs.<sup>14</sup> The infection frequently persists and might develop into a chronic infection.<sup>1,6</sup> Persistent infection by human parvovirus B19 (HPVB19) is well known in human medicine, manifesting in various disease forms, and giving rise to subclinical infection. The manifestation of the disease in HPVB19 viral infection depends on age, sex, and the immunological and hematological status of the patient.<sup>16</sup> So far, the immunological mechanisms underlying the transition from subclinical EqPV-H infection to clinically relevant hepatitis have not been elucidated in horses. The routes of transmission, apart from iatrogenic transmission through administration of equine biological products contaminated with EqPV-H, are not fully understood. Interestingly, EqPV-H DNA could be detected in several equine samples (such as serum, buccal, nasal, fecal, and semen) and viremia occurred in horses with or without a history of equine biologic product administration, suggesting additional transmission routes.<sup>1,14,17</sup> Viremia and hepatitis was demonstrated in horses inoculated with EqPV-H DNA (contaminated equine biological products) via the bloodstream and intranasally,<sup>14</sup> but not perorally.<sup>18</sup> Evidence is lacking for vertical, venereal, and vector transmission.<sup>14,17</sup> Although, EqPV-H infection can frequently occur among horses, the incidence of Theiler's disease after EqPV-H infection is low (1.4%–2.2%),<sup>1</sup> but case fatality rate can be high (81.5%).<sup>19</sup> For this reason, we aimed to determine the frequency of subclinical viremia of EqPV-H among hospitalized horses and to investigate potential shedding routes that might put other horses at risk for hospital-acquired infection. We hypothesized that EqPV-H infection would be frequently detected among hospitalized horses and potential shedding might occur through feces, nasal and buccal secretions.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection and study design

In this cross-sectional study, 116 horses hospitalized at the Equine Hospital of the University of Veterinary Medicine Vienna between February 2021 and March 2022 that were not presented for hepatic disease were included. To detect any seasonality, we aimed to include at least 25 horses for each season (spring, summer, autumn, and winter). From all horses, undergoing blood collection for diagnostic purposes, 4 mL of whole blood were taken in a blood collection tube without anticoagulant (serum tube), and if feasible, feces, nasal, and buccal swab samples were also collected in tubes without additives. To assess the viral status of horses on admission, samples were collected within the first 24 h of hospitalization. Until further analysis,

collected samples were stored at  $-20^{\circ}\text{C}$  initially and then transferred to  $-80^{\circ}\text{C}$ . All blood samples were analyzed by luciferase immunoprecipitation system (LIPS) assay for detection of EqPV-H antibodies and by PCR to detect viremia. Further samples (feces, nasal, and buccal secretions) of viremic horses (as defined in section 2.3 below) were analyzed by PCR for EqPV-H DNA detection.

### 2.2 | Anti-EqPV-H antibody detection by LIPS

Anti-EqPV-H viral protein 1 (VP1) antibodies were semi-quantitatively measured using the LIPS assay as previously described.<sup>6,20</sup> Briefly, we produced a recombinant Renilla luciferase VP1 fusion antigen expressed from a pREN2 vector in Cos-1 cells. Anti-VP1 antibodies were then measured by mixing 10  $\mu\text{L}$  serum samples (1:10 diluted in buffer A–50 mM Tris [pH 7.5], 100 mM NaCl, 5 mM MGCL<sub>2</sub>, and 1% Triton X-100) with the VP1 fusion antigen and precipitating on Filter HTS plates (Millipore, Bedford, MA) using a 30% suspension of Ultralink protein A/G beads (Pierce Biotechnology, Rockford, IL). Signal was quantified as relative light units (RLU) in technical duplicates using a plate luminometer (LB 960 XS3; Berthold, Bad Wildbad, Germany). Samples were defined as positive if their mean RLU value was greater than the mean RLU of the negative control, plus 3 standard deviations.

### 2.3 | EqPV-H-DNA determination by quantitative and nested polymerase chain reaction

Viral DNA of serum, nasal, and buccal swab samples was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Cat. No 52904, Hilden, Germany) according to the manufacturer's recommendations. Nasal and buccal swab samples underwent a few modifications included in Data S1. The fecal samples were extracted with Qiagen AllPrep PowerFecal Pro DNA/RNA Kit (Cat. No 80254, Hilden, Germany) according to the manufacturer's recommendations. Extracted DNA was directly used for qPCR or stored at  $-80^{\circ}\text{C}$  until further analyses. As described previously, EqPV-H- and TTC17-specific primers and probes were used for qPCR screening, while the TTC17 assay was slightly adapted for use as a duplex assay (Table 1).<sup>21</sup> The protein coding gene TTC17 is a single copy gene per haploid nuclear genome, existing in mammalian species, and was used as an isolation control in this assay.<sup>22</sup> Detailed information regarding the oligonucleotide sequences of the primers, probes, and the EqPV-H positive control are shown in Table 1.

Quantitative PCR on serum samples was performed as previously described,<sup>21</sup> with minor modifications; details can be found in the Data S2. To calculate the viral load for the EqPV-H qPCR assays, standard curves were generated using serial dilutions of a synthetic oligonucleotide ( $10^6$  to  $10^1$  copies per reaction). Viral load, stated as genome equivalents (GE) per reaction, was calculated with the formula  $N_n = 10^{((n-b)/m)}$  ( $n$  = Ct value of the sample,  $b$  = offset,  $m$  = slope) by interpolation to the respective standard curve. Viral load by GE per ml serum, per gram feces, or per swab were then

**TABLE 1** Quantitative and nested PCR oligonucleotide sequences used to detect equine parvovirus hepatitis (EqPV-H) and tetratricopeptide repeat domain 17 (TTC17).

Assay	5'to 3' Sequence of oligonucleotide	Amplicon size (bp)
EqPV-H qPCR <sup>21</sup>	Forward primer: 5'-AAG ATA TGC CGC CAT TTG AA-3' Reverse primer: 5'-CTG AAA AGG CAT TCC GTC AG-3' Probe: 5'-FAM-CAG AGA AAT/ZEN/CCT GAG CGG TGG CCT-IBFQ-3 Positive control: ATC TTC TAT AAA GAT ATG CCG CCA TTT GAA AAG GCC ACC GCT CAG GAT TTC TCT GAC TAT TAT GTT TCT GAC GGA ATG CCT TTT CAG ACT TTG TAT G	77
TTC17 qPCR <sup>a</sup>	Forward primer: 5'-CTG GAC AAC AGC CAT GAC AAA-3' Reverse primer: 5'-GGG TCC TCC TCT GCT CCT GT-3' Probe: 5'-YakYel-CAAG+TC+AC+AG+TC+AC+C-IBFQ-3'	147
EqPV-H Nested PCR <sup>1</sup>	Forward primer first round: 5'-GGA GAA GAG CGC AAC AAA TGC A-3' Reverse primer first round: 5'-AAG ACA TTT CCG GCC GTG AC-3' Forward primer second round: 5'-GCG CAA CAA ATG CAG CGG TTC GA-3' Reverse primer second round: 5'-GGC CGT GAC GAC GGT GAT ATC-3'	452 433

Note: "+" stands for affinity plus locked nucleic acid nucleotides.

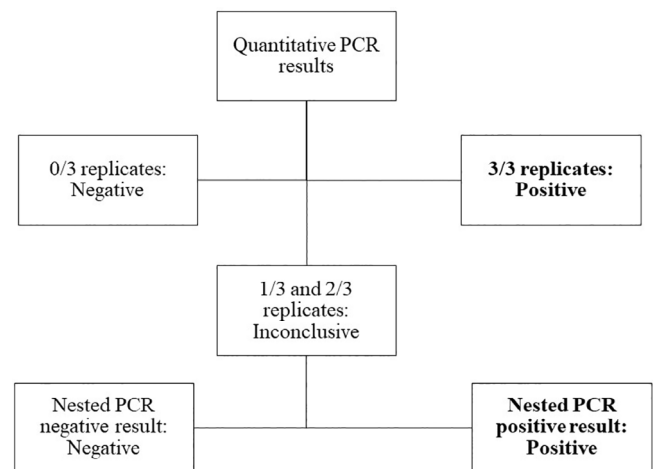
<sup>a</sup>Adapted from Zehetner et al.

calculated considering the amount used for isolation and the elution volume used according to the different isolation protocols.

Samples were categorized as positive when EqPV-H DNA was detected in 3/3 qPCR replicates and negative when no viral DNA was detected in any qPCR replicate. Samples that were inconclusive by qPCR result with only 1/3 or 2/3 positive qPCR replicates, were validated by nested PCR (nPCR). According to their nPCR result they were re-categorized as positive or negative (Figure 1). The nPCR consisted of 2 primer pairs targeting the NS gene, as reported previously (Table 1)<sup>1</sup> with slight modifications; details can be found in the Data S3. Amplimers were analyzed by 1% agarose gel electrophoresis. Representative amplimers of expected size were sequenced (Microsynth) and compared to the EqPV-H NCBI reference sequence (NC\_040652.1) using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to confirm presence of EqPV-H DNA in the samples.

## 2.4 | Statistics

Effects on the categorization of horses as EqPV-H negative (0) or positive (1) were evaluated via a binary logistic regression model (function *glm*) in R (R version 4.1.2).<sup>23</sup> The categorization was fitted as binary response and the age (in years), breed (Warmblood, other breeds), sex (gelding, mare, stallion), and season (spring, summer, autumn, winter) were fitted as fixed effects. Multicollinearity between the fixed effects was evaluated via variance-inflation factors (package *car*, version 3.1-1, function *vif*).<sup>24</sup> No multicollinearity was observed. Pairwise comparisons between the levels of each categorical predictor were conducted via the estimated marginal means (package *emmeans*, version 1.8.5, function *emmeans*).<sup>25</sup> Pairwise comparisons between the levels of sex and season were corrected for multiple testing separately for each predictor via the Bonferonni-Holm method (option



**FIGURE 1** EqPV-H DNA detection categorization by PCR results. Samples were categorized as positive when EqPV-H DNA was detected in all three quantitative PCR (qPCR) replicates while samples lacking viral DNA in all replicates were classified as negative. Samples where qPCR results were inconclusive with only one or two replicates positive out of three, underwent validation using nested PCR (nPCR). Subsequent categorization as positive or negative depended on the nPCR outcome.

"adjust = 'holm'" in the function *emmeans*). We declared significance at an alpha cut off of 5%.

## 3 | RESULTS

### 3.1 | Study cohort and collected samples

The study included a total of 116 horses of various breeds, including Warmblood (most presented breed, 82/116), Haflinger, Arabian, Pony,

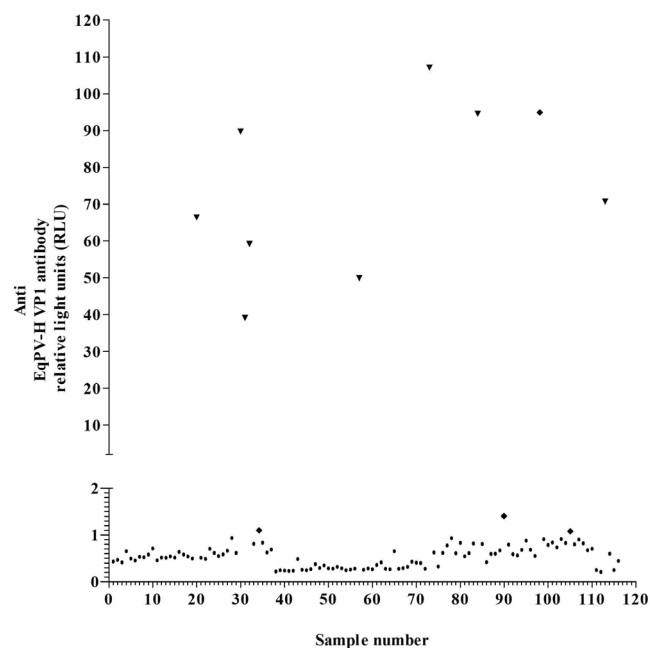
Icelandic, Quarter Horse, and Draft Horse. Among these, there were 34 mares, 71 geldings, and 11 stallions. The age of the cohort ranged between 1 and 32 years (median 13). Across the 4 seasons, equids were evenly distributed with 27, 35, 26, and 28 individuals observed during spring, summer, autumn, and winter, respectively. In total 116 sera, 103 fecal samples, 107 nasal swabs, and 107 buccal swabs were collected.

### 3.2 | Anti-EqPV-H VP1 seroprevalence among hospitalized horses

The seroprevalence of the hospitalized horses was 10.3%, with 12 out of 116 samples positive for anti-EqPV-H VP1 antibodies as detected by the LIPS assay (Figure 2).

### 3.3 | Subclinical infection and EqPV-H DNA detection in fecal, nasal, and buccal samples

Of the 116 serum samples that were screened with both a duplex q-PCR assay for EqPV-H and TTC17 DNA, TTC17 DNA was detected in all samples, thus confirming successful DNA isolation. EqPV-H DNA was detected in 3/3 replicates in 8 samples, 2/3 replicates in



**FIGURE 2** Anti-equine parvovirus-hepatitis VP1 seroprevalence. Luciferase immunoprecipitation system (LIPS) value in relative light units (RLUs) for each of the serum samples. Triangle marking indicating horses where EqPV-H DNA and EqPV-H antibodies were detected ( $n = 8$ ), diamond marking indicating horses where no EqPV-H DNA, but only EqPV-H antibodies were detected ( $n = 4$ ), and dot marking indicating absence of EqPV-H antibody detection.

6 samples, and 1/3 replicates in 19 samples of serum. As many samples were inconclusive, potentially as they were close to the detection limit of the qPCR assay, an nPCR was carried out for further validation of the qPCR results of all samples with 1/3 and 2/3 qPCR replicate positive. Nested PCR confirmed all of the 2/3, as well as in 1 1/3 replicate positive sera of horses. Sequencing of 4 representative amplicons from 1/3 or 2/3 qPCR replicate positive samples (sample 15, 22, 63, 107) showed 99%–100% nucleotide sequence identity to EqPV-H NS1. Based on the PCR results and the categorization mentioned earlier, 15 serum samples were classified as positive and 18 samples, which were inconclusive by qPCR and negative by nPCR were classified as negative finally.

According to qPCR results of the serial dilution of the synthetic oligonucleotide, which was also used as positive control, the maximum viral load in these subclinical infected horses was set at  $1.3 \times 10^6$  GE/mL serum. However, the viral load in general was rather low and not quantifiable in many of the samples of this cohort. The limit of quantification of the qPCR assays was below 10 GE per reaction, resulting in a limit of quantification of  $1.8 \times 10^4$  GE/mL in serum,  $1.4 \times 10^4$  GE/g in feces, and  $1.7 \times 10^3$  GE/swab in swab samples. Of the 15 EqPV-H DNA positive horses, only 5 serum samples were quantifiable, ranging between  $6.7 \times 10^4$  and  $1.3 \times 10^6$  GE/mL serum.

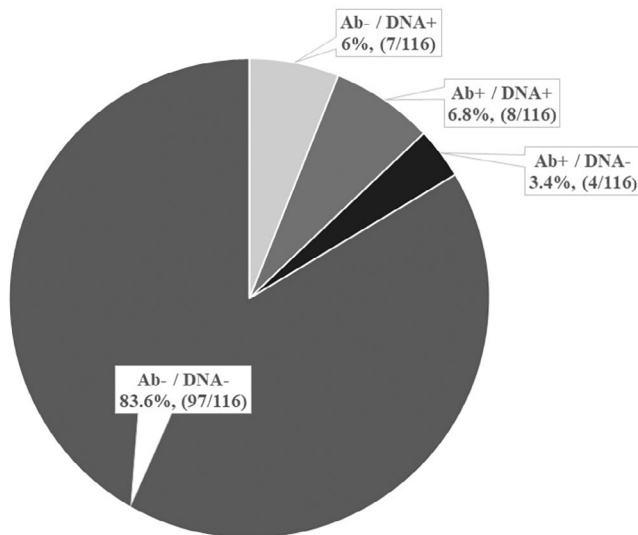
Further available samples (fecal, nasal, and buccal samples) of the 15 EqPV-H DNA positive horses were screened for EqPV-H DNA by qPCR. Two nasal and 1 fecal sample were positive by qPCR results (3/3 replicates positive). Five fecal, 5 nasal and 4 buccal samples were inconclusive by qPCR (1/3 or 2/3) and negative by nPCR, and were therefore categorized as negative finally. According to qPCR the 2 nasal swab samples (horse 57 and 113) were in the quantifiable range with  $3.2 \times 10^3$  and  $2.3 \times 10^3$  GE/swab (details can be found in Table S1).

### 3.4 | LIPS and quantitative PCR data

Horses were assigned to 4 groups according to PCR categorization (positive, negative) and anti-EqPV-H VP1 antibody results (positive, negative), as illustrated in Figure 3. Ninety seven samples of 116 (83.6%) were PCR and anti-EqPV-H VP1 antibody negative whereas 8 samples (6.8%) were positive on both. Seven samples (6%) were only positive on PCR and 4 samples (3.4%) only positive for anti-EqPV-H VP1 antibody.

### 3.5 | Possible risk factors affecting EqPV-H infection

No effects of age ( $P$ -value = 0.438), breed ( $P$ -value = 0.96), sex ( $P$ -value  $\geq 0.29$ ), and season ( $P$ -value  $> 0.72$ ) on EqPV-H infection status were detected (detailed results and additional information can be found in Table S2).



**FIGURE 3** Group designation of horses based on EqPV-H antibody detection [present (Ab+) or absent (Ab-)] and EqPV-H DNA detection [present (DNA+) or absent (DNA-)].

## 4 | DISCUSSION

This study demonstrated evidence of current or previous infection of EqPV-H in this hospitalized cohort with a seropositivity of 10.3% (12/116). Overall, we detected a low EqPV-H DNA viral load in 12.9% (15/116) of horses. Out of the 15 viremic horses, only 5 of the serum samples (4.3%) were above the limit of quantification. Furthermore, in order to improve understanding of EqPV-H potential transmission routes, we collected additionally feces, nasal, and buccal swab samples. EqPV-H DNA was detected and categorized as positive in 2 nasal swab and 1 fecal sample of viremic horses. It is crucial to be aware of these potential shedding routes since nasal EqPV-H infection has been previously reported<sup>18</sup> and this result might imply the potential risk of nosocomial EqPV-H infection among hospitalized horses. Considering the fact that hospitalized horses might be immunocompromised due to systemic disease or being stressed due to transportation, conditions that might negatively impact their immunological status, there could be an increased risk of EqPV-H infection in this cohort.

The seroprevalence in this study was 10.3% (12/116) and we detected 9 samples with clearly positive antibody results, while 3 additional samples were close to the detection threshold where no conclusions can be drawn regarding the measured antibody level and associated immunologic status.<sup>6,7</sup> The seroprevalence in this study (10.3%) was lower than the lowest seroprevalence of 15% (15/100)<sup>1</sup> reported in the United States and much lower than the highest seroprevalence of 34.6% (136/392) that was reported in Europe mostly from racing horses.<sup>15</sup> A much higher seropositivity was also detected in another study from Austria (30.1%; 78/259), while in both these studies the same LIPS assay was used and, interestingly, the horses were housed in similar regions.<sup>7</sup> In our study, viremia occurred in 12.9% (15/116) of horses sampled, which is within the reported range of 3.2%–19.8%, when compared to other studies worldwide.<sup>1,4–9,11,12,17,26</sup>

Although another study carried out from horses housed at similar region as in our study, reported a lower EqPV-H DNA prevalence (by nPCR) of 8.9% (23/259).<sup>7</sup> These findings indicate the variability of EqPV-H occurrence among different cohorts, even if they are housed in similar regions. Horses included in our study consisted mostly of leisure horses that spent their lifetime mostly in 1 regional area. This is in comparison to horses under high performance training with suspected increased national and international travel. It is reasonable to assume that increased travel might increase the probability of EqPV-H infection (by contact with new premises, other horses, etc.).

The variation in detectable viremia and antibody prevalence might be associated with individual or epidemiological differences.<sup>7,14,27</sup> Several studies reported that EqPV-H viremia occurred mostly in elderly horses,<sup>7,8,28,29</sup> whereas another study reported the occurrence particularly in young racing horses (average 4.4 years old).<sup>8</sup> In our study, no statistically significant increased risk for EqPV-H infection in aged horses was detected. When comparing our study cohort to racing horses, where an increase of EqPV-H infection might be expected as mentioned previously, a much lower and variable prevalence of viremia with 4.4% (14/132, by nPCR) in South Korea in late spring,<sup>8</sup> 8.3% (5/60, by nPCR) in China from winter,<sup>4</sup> and in Germany with 7.14% (28/392 by qPCR) without information on season have been reported.<sup>5</sup> A study from an isolated study cohort in Germany revealed a much higher prevalence of EqPV-H frequency (19.8%, 16/81, by qPCR) without information on season when compared to sampling after 5 years (5%, 4/80) in winter.<sup>6</sup> The effect of season has been previously discussed for Theiler's Disease, in that it tends to occur in the warmer months.<sup>14,26,28</sup> In contrast, subclinical infection of EqPV-H can be seen almost all year round as seen in our study and according to the studies stated above.

We detected 7 viremic horses without antibodies, which might indicate an acute infection, or persistent infection with compromised immune system, immune invasion of the virus or low antigenic stimulation caused by low levels of viral load, which was not enough to elicit an appropriate immune response for antibody production. In a few studies investigating EqPV-H DNA prevalence and seroprevalence,<sup>1,5</sup> only 1 study identified 2 viremic horses without antibodies.<sup>7</sup> In humans infected with HPV B19, the absence of antibodies in the presence of a viral load can be attributed to antibodies being below the assay threshold or to virus-antibody complexes forming, which could be the case in our study.<sup>30</sup>

In the present study, 18 serum samples, 5 fecal, 5 nasal and 4 buccal swab samples were categorized as inconclusive by qPCR and could not be validated by nPCR. Due to the missing validation, they were categorized as negative, while they could have retained inconclusive result in qPCR due to a very low viral load present in the samples. Interestingly, an experimental EqPV-H infection study reported 1 horse with low viral load upon retesting after 13 weeks despite clearing viremia 9 weeks after inoculation.<sup>14</sup> It is possible that horses might harbor a low viral load of EqPV-H that is around the limit of detection due to a chronic/persistent infection.<sup>6</sup> In humans, parvovirus B19-DNA can be detected after years of initial infection and differentiating between infective and non-infective parvovirus B19 is

essential for determining the cause of the disease. It is well known, that serum PCR cannot differentiate between parvovirus B19 DNA remnants released for example, by persistently infected cells and viral DNA produced from infectious virions by viral replication.<sup>31</sup> This might also be the case in equids where non-infectious particles might result in low-level viremia around the limit of detection.<sup>7,13</sup> The used viral DNA detection technique and its interpretation affects the outcome of different studies and might also explain differences in EqPV-H prevalence. For example, nested PCR uses 2 pairs of oligonucleotides allowing more cycles in order to increase sensitivity of the PCR but it also increases the likelihood of contamination. In contrast, qPCR is very sensitive and specific due to the probe based design of the assay we used in this study and provides the opportunity to determine the viral load and to perform multiplex assays in order to amplify several targets in a single reaction.<sup>32</sup> However, interpretation of qPCR results might be difficult for samples with low viral load, as demonstrated in our study, therefore, nPCR was used for additional validation. Still, it might be relevant to keep the high amounts of inconclusive samples in mind as they might reflect that even more horses potentially harbor very low amounts of EqPV-H DNA in sera, feces, nasal and buccal secretions. However, as these findings were inconclusive and not validated by nPCR, the relevance of this low viral amounts is questionable, and they have to be evaluated very critically. Therefore, they were mentioned, but excluded from further analysis.

Currently, iatrogenic transmission through biologic products (whole blood, hyperimmune plasma, tetanus/botulism antitoxin, allogeneic bone-marrow-derived mesenchymal stromal cells, *Streptococcus equi* antiserum, pregnant mares serum) has been demonstrated in EqPV-H infection, but understanding other potential routes of transmission is in its infancy.<sup>14</sup> Interestingly, evidence exists where healthy horses, without history of receiving any equine biologic blood product but having been in contact with Theiler's diseased cases, developed EqPV-H infection, further suggesting horizontal transmission routes.<sup>14,15,26,28</sup> In our study we observed that the horse with the highest viral load in the serum showed the presence of EqPV-H DNA in both fecal and nasal swab sample, while the other EqPV-H DNA positive nasal swab sample was collected from another horse with rather higher viral load in serum compared to the other horse in the study. An experimental study reported a peak fecal viral load of  $7.09 \times 10^3$  GE/swab whereas our study detected low viral load in the fecal sample which was under the quantification limit.<sup>14</sup> In a healthy cohort with no further information regarding the viral load, EqPV-H DNA in nasal secretions was detected in 1 viremic horse (out of 87 horses),<sup>13</sup> and in 3 viremic horses (out of 102 horses) in another study.<sup>17</sup> The detected viral load in the nasal secretions ( $2.3 \times 10^3$  GE/swab) of the viremic horses in our study was much lower in comparison to an experimental study with detection of peak viral load in nasal secretion of  $2.87 \times 10^6$  GE/swab.<sup>14</sup> Recently, a study demonstrated EqPV-H infection of horses by nasal inoculum (viral load of  $5 \times 10^6$  GEs of EqPV-H), but not by oral route.<sup>18</sup> This raises the question of a possible horizontal EqPV-H transmission by natural shedding of EqPV-H via nasal secretions or feces. However, it is important to

consider that there is no clear information regarding the amount of virus, which is required for infection. Our study detected a low viral DNA load in the tested samples, where no statement can be made as to whether the detected viral DNA indicated the presence of infectious viral particles or not. Infection studies addressing this issue would be of interest in the future.

One-point sampling provided no opportunity to obtain information regarding the time frame of viremia or shedding in the viremic horses that might have an impact on the clinical relevance of chronic or persistent infections, which limited this study.

In this study on EqPV-H infection among hospitalized horses, we obtained epidemiological information regarding the frequency of EqPV-H viremia and seroconversion. It was demonstrated that EqPV-H DNA can be detected in serum, feces, and nasal secretion of hospitalized horses, and so, it can be assumed that EqPV-H shedding might occur, which could pose a risk of horizontal transmission. Important to note that parvoviruses are non-enveloped and therefore extremely stable under environmental conditions,<sup>33</sup> a fact that might favor transmission. Since there are horses in early or subclinical viremia or which are persistent/chronically infected, it is advisable to screen for the EqPV-H virus in commercial biological products, but even more importantly, before plasma and blood transfusions carried out among hospitalized horses, where commercial blood products are not available. No conclusion can be made regarding infection, prevention, and control measurements for infected horses at this time, but it is highly recommended to adhere to hospital hygiene regulations.

#### CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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#### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The ethics committee of the University of Veterinary Medicine Vienna (study reference number ETK-124/08/2020) approved the sample and data collection.

**OFF-LABEL ANTIMICROBIAL DECLARATION**

Authors declare no off-label use of antimicrobials.

**HUMAN ETHICS APPROVAL DECLARATION**

Authors declare human ethics approval was not needed for this study.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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