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Short Communication

Frequency of Detection of Respiratory Pathogens in Nasal Secretions From Healthy Sport Horses Attending a Spring Show in California

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ABSTRACT

The objective of this study was to determine detection frequency of respiratory viruses (equine influenza virus [EIV], equine herpesvirus-1 [EHV-1], EHV-2, EHV-4, EHV-5, equine rhinitis A virus [ERAV], ERBV) and bacteria (*Streptococcus equi* ss. *equi*[*S. equi*], *S. equi* ss. *zooepidemicus*[*S. zooepidemicus*]) in 162 nasal secretions and 149 stall swabs from healthy sport horses attending a spring show in California. Nasal and stall swabs were collected at a single time point and analyzed using qPCR. The detection frequency of respiratory pathogens in nasal secretions was 38.9% for EHV-2, 36.4% for EHV-5, 19.7% for *S. zooepidemicus*, 1.2% for ERBV, 0.6% for *S. equi* and 0% for EIV, EHV-1, EHV-4 and ERAV. The detection frequency of respiratory pathogens in stall swabs was 65.8% for *S. zooepidemicus*, 33.5% for EHV-2, 27.5% for EHV-5, 3.3% for EHV-1, 1.3% for EHV-4 and 0% for EIV, ERAV, ERBV and *S. equi*. Commensal viruses and bacteria were frequently detected in nasal secretions and stall swabs from healthy sport horses. This was in sharp contrast to the subclinical shedding of well-characterized respiratory pathogens. Of interest was the clustering of five EHV-1 qPCR-positive stalls from apparently healthy horses with no evidence of clinical spread. The results highlight the role of subclinical shedders in introducing respiratory pathogens to shows and their role in environmental contamination. The results also highlight the need to improve cleanliness and disinfection of stalls utilized by performance horses during show events.

1. Introduction

Outbreaks are one of the worst outcome of any equestrian event. Unfortunately, the equine industry has experienced quite some dramatic outbreaks in recent years, most of them associated with EHV-1 [1,2]. Circumstances surrounding these outbreaks remain often elusive; however, various factors have consistently highlighted population size and absence of biosecurity protocols as key predisposing factors for the rapid spread of respiratory infections [1,3,4]. The population of equids most commonly affected by upper respiratory tract infections are young performance horses, emphasizing the risk of movement and commingling in regard to transmission of respiratory pathogens [5,6]. While equids with acute onset of fever and respiratory signs are easily recognized and isolated to prevent disease spread, silent shedders represent a significant source of transmission for susceptible horses and of environmental contamination. The rate of silent shedders is highly dependent on population age and use as well as specific pathogen tested, with reported frequencies ranging from 0% to 4% [7–13]. It has become imperative to determine the frequency of silent shedders for large multiday equestrian events and their impact on environmental contamination in order to customize monitoring and preventive strategies aimed at reducing the risk of pathogen transmission. Therefore, the objective of the present study was to investigate the presence of selected respiratory pathogens in nasal

Abbreviations: EHV, Equine herpesvirus; EIV, Equine influenza virus; ERV, Equine rhinitis virus; qPCR, quantitative real-time polymerase chain reaction.

Ethical/Animal welfare statement: There were no animal welfare issues in the generation of the study data.

Conflict of Interest statement: Dr. Pusterla, Dr. Sandler-Burtness, Mrs. Barnum, Mrs. Hill, Mr. Portener, Mrs. Ridland and Dr. Schumacher have no conflict of interest to declare. Mr. Mendonsa and Mr. Khan work for Fluxergy.

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secretions and stall swabs collected from healthy sport horses attending a multi-day spring show in California.

2. Materials and Methods

2.1. Study population

The study population was composed of 162 out of a total of 320 adult, healthy sport horses attending a multi-day hunter/jumper show event in southern California (April 12–16, 2022). The enrollment occurred on a voluntary basis and written owner's consent was acquired prior to any sample collection. The show represented the first equestrian event following the resolution of a multicounty EHM outbreak in the spring of 2022. Horses attending the show had to comply with the following requirements: isolation at home barn for at least 28 days, USEF return-to-competition protocols (https://www.usef.org/media/press-releases/return-to-competition-declaration-forms-ehv-1), horse health declaration form (https://theplacetojump.com/equine-health-biosecurity), equine certificate of vaccination (https://secureservercdn.net/198.71.233.229/af7.cb6.myftpupload.com/wp-content/uploads/2021/07/USEF-equine-vaccination-record.pdf) and one negative EHV-1 qPCR test within 28 days of the event.

2.2. Sample collection and analysis

Nasal swabs were collected on 2 consecutive days (April 14 and 15, 2022), representing the third and fourth show day. Stall swabs were collected 2 days after the show ended and horses vacated the stalls. Wearing disposable gloves, the attending veterinarian collected nasal secretions from the rostral nasal passages using one 6" rayon-tipped swab (Puritan Sterile Rayon Tipped Applicators, Guilford, ME, USA). The swabs were placed in 10 mL evacuated blood tubes (BD Vacutainer, Franklin Lakes, NJ, USA) without any added solution. Stall swabs were collected using sponges soaked in neutralizing buffer (3M, St. Paul, MN). Collection of environmental samples included the swabbing of the inside of the stall door, stall walls, rim of the feeder and/or water bucket if available. In order to prevent possible cross-contamination during sample collection and handling, the collection of stall swabs followed stringent biosecurity protocols. Nasal and stall swabs were kept refrigerated and shipped on ice to the laboratory for sample processing and analysis.

Nucleic acid extraction from nasal and stall swabs was performed 24 hours postcollection using an automated nucleic acid extraction system (QIAcube HT, Qiagen, Valencia, CA, USA) according to the manufacturer's recommendations. The swabs were assayed for the presence of equine influenza virus (EIV), equine herpesvirus-1 (EHV-1), EHV-2, EHV-4, EHV-5, equine rhinitis A virus (ERAV), ERBV, *Streptococcus equi* ss. *equi* (*S. equi*) and *S. equi ss. zooepidemicus* (*S. zooepidemicus*) using previously reported real-time TaqMan PCR assays [5,14,15].

2.3. Data analysis

Demographic information (age, breed, sex) from the study horses was evaluated using descriptive analyses. All statistical analyses were performed using Stata Statistical Software (College Station, TX, USA), and statistical significance was set at $P < .05$.

3. Results

Nasal swabs were collected from 162 adult, healthy sport horses. The horse population was composed of 130 geldings, 28 mares and 4 stallions, ages 1–32 years of ages (median age of 11 years). The population included 153 Warmbloods and 9 ponies. The horses were stabled in 14 different barns, with a median number of 10 horses per barn (range 5–39 horses per barn). The qPCR detection of respiratory pathogens in nasal secretions was as follows: 63 qPCR-positive nasal swabs for EHV-2 (38.9%), 50 qPCR-positive for EHV-5 (36.4%), 32 qPCR-positive for *S. zooepidemicus* (19.7%), 2 qPCR-positive ERBV (1.2%), and 1 qPCR-positive for *S. equi* (0.6%; Table 1). EIV, EHV-1, EHV-4, and ERAV were not detected by qPCR in any of the nasal swabs. The 3 horses with detectable respiratory pathogens with known association to clinical disease (ERBV, *S. equi*) originated from two different barns (Table 2).

Environmental swabs were collected from 149 stalls. Thirteen stalls in barn N were not swabbed because they had not been vacated at the time of sample collection. The qPCR detection of respiratory pathogens in stall swabs was as follows: 98 qPCR-positive nasal swabs for *S. zooepidemicus* (65.8%), 50 qPCR-positive for EHV-2 (33.5%), 41 qPCR-positive for EHV-5 (27.5%), 5 qPCR-positive for EHV-1 (3.3%), and 2 qPCR-positive for EHV-4 (1.3%; Table 1). EIV, ERAV, ERBV, and *S. equi* were not detected by qPCR in any of the stall swabs. The two EHV-4 qPCR-positive stall swabs originated from 2 different barns, while the 5 EHV-1 qPCR-positive stall swabs all clustered in the same barn (Table 2, Fig. 1).

There was no agreement in the molecular detection of true respiratory pathogens (EHV-1, EHV-4, *S. equi*, ERBV) between individual nasal swabs and corresponding environmental samples. This was in sharp contrast to the detection of lesser-characterized respiratory pathogens (EHV-2, EHV-5, *S. zooepidemicus*). For *S. zooepidemicus* molecular agreement between the two sample types was 40.9%. For EHV-2 and EHV-5, the overall molecular agreement between individual nasal swabs and environmental samples was 63.7% and 64.4%, respectively.

4. Discussion

The present study results showed that shedding of well-characterized equine pathogens (EIV, EHV-1/-4, ERVs, *S. equi*) was a relatively uncommon finding as only 3 out of 162 horses tested qPCR-positive for ERBV and *S. equi*. This study population was unique as the collection of nasal swabs occurred at the tail end of a 28-day suspension of shows in California due to a multicounty EHM outbreak in the spring of 2022. The results are not surprising, considering that movement and commingling are key factors in the transmission of contagious respiratory pathogens [16]. All study horses were considered healthy at arrival to the show and remained healthy throughout the show period. The data highlight the occasional detection of subclinical shedders and emphasizes the risk that these horses represent in introducing contagious respiratory viruses and bacteria into a population of susceptible horses. Subclinical shedding of *S. equi* is a well-recognized status that often occurs weeks postnatural infection. These so-called long-term, subclinical *S. equi* carriers can become a source of new or recurrent disease in susceptible horses [17,18]. A similar situation occurs with viral respiratory pathogens, although, the shedding period is often shorter compared to horses experiencing clinical disease. Primary infections with ERBV have been reported mainly in weaning foals and young horses entering training centers, while adult horses often display no to short and self-limiting signs [19]. Apparently, none of the three horses with detectable respiratory pathogens nor the other show horses developed clinical disease during the show period. While, this observation does not negate the risk of transmission, it highlights that in a population of healthy show horses, respiratory pathogens with little clinical relevance do occasionally circulate. This was in sharp contrast to the detection of commensal respiratory viruses (EHV-2/-5) and bacteria (*S. zooepidemicus*). The two gamma herpesviruses EHV-2 and EHV-5 are widely spread in horse populations and can be found in healthy horses as well as in horses with respi-

Table 1
qPCR results for equine respiratory pathogens in nasal and stall swabs from healthy sport horses.

Pathogen	Nasal Swabs (n = 162)	Stall Sponges (149)	Agreement (%) (149)
EIV	0	0	0
EHV-1	0	5	0
EHV-2	63 (56 + 7)	50	63.7
EHV-4	0	2	0
EHV-5	50 (45 + 5)	41	64.4
ERAV	0	0	0
ERBV	2	0	0
S. equi	1	0	0
S. zooepidemicus	32	98	40.9

The results are reported as number of qPCR-positive nasal swabs and stall sponges. One hundred and forty nine horses had nasal and stall swabs collected, while for 13 horses only nasal swabs were collected. The results in parenthesis reflect the distribution between the two horse groups for each respiratory pathogen. Further, overall molecular agreement between nasal and stall samples is listed for each respiratory pathogen.

Table 2
Detection of respiratory pathogens in nasal swabs and stall sponges collected from healthy sport horses according to barn and stall number.

Barn number	Stall number	qPCR results	
		Horse nasal swab	Stall swab
P	14	**ERBV**, *S. zooepidemicus*, EHV-5	*S. zooepidemicus*, EHV-2, EHV-5
CS-C	7	***S. equi***, *S. zooepidemicus*	*S. zooepidemicus*, EHV-2
CS-C	9	*S. zooepidemicus*, EHV-2	**EHV-4**, *S. zooepidemicus*, EHV-2
CS-C	28	*S. zooepidemicus*, EHV-5	**EHV-1**, *S. zooepidemicus*, EHV-2, EHV-5
CS-C	30	EHV-2	**EHV-1**, *S. zooepidemicus*, EHV-2, EHV-5
CS-C	41	**ERBV**, EHV-2, EHV-5	Negative
CS-C	63	EHV-5	**EHV-1**, *S. zooepidemicus*, EHV-2, EHV-5
CS-C	64	EHV-2, EHV-5	**EHV-1**, *S. zooepidemicus*, EHV-2, EHV-5
CS-C	68	EHV-2	**EHV-1**, *S. zooepidemicus*, EHV-2
CS-A	14	EHV-2	**EHV-4**, *S. zooepidemicus*, EHV-2, EHV-5

Relevant respiratory pathogens with recognized clinical disease are listed in bold.

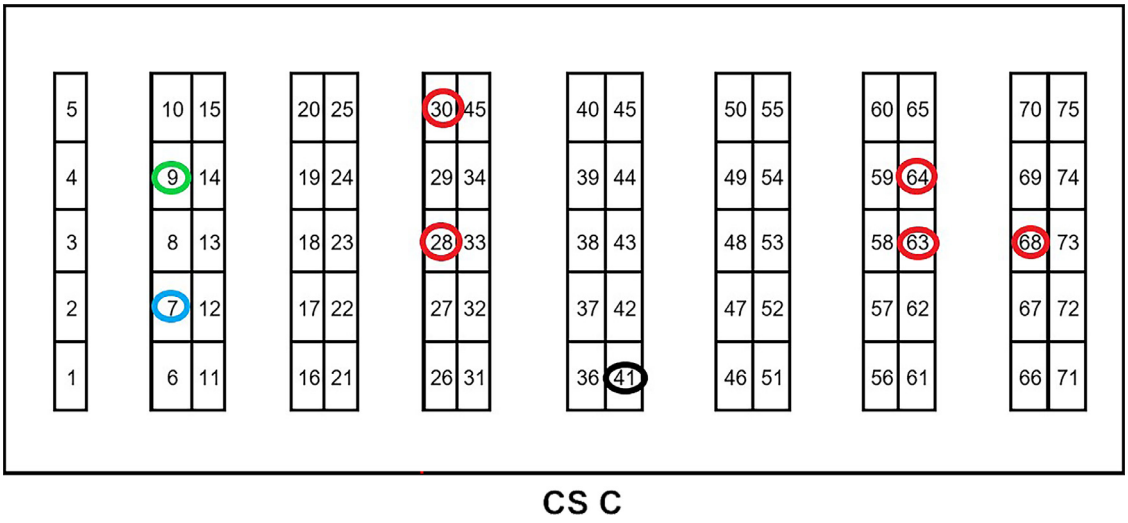

Fig. 1. Layout of Barn CS-C showing EHV-1 qPCR-positive stalls (red circles), EHV-4 qPCR-positive stalls (green circle), and location of *S. equi* qPCR-positive (blue circle) and ERBV qPCR-positive (black circle) horses. The second EHV-4 qPCR-positive stall in barn CS-A and the second ERBV-qPCR positive horse in barn P are not shown in the figure.

ratory disease [20]. Frequency of detection of EHV-2 and EHV-5 in nasal secretions of healthy equids has been previously reported to range between 3% and 74% [10,21–23]. The detection of *Streptococcus zooepidemicus* in 19.7% of the study population was lower than previously reported with detection frequencies in the upper airways of 75.1%–87.5% [11,24]. While the colonization of the upper airway with commensal viral and bacterial pathogens is apparently high and population dependent, their temporal monitoring in show horses could potentially be used to assess risk of transmission. Of interest was the lack of association between nasal secretions and environmental swabs for well-characterized respiratory pathogens. The cluster of 5 EHV-1 qPCR-positive stall sponges originating from the same barn most likely reflect silent and active spread. The origin of EHV-1 is speculative but likely originated from a focal source and slowly spread thereafter directly via horse-to-horse contact or indirectly via contaminated equipment. Previous studies have shown that silently infected horses shed EHV-1 for a short period, which could explain the inability to detect EHV-1 in the horses occupying the positive stalls [25]. While EHV-1 is routinely detected in upper airway secretions of

infected horses, it has also been detected in feces, potentially contributing to environmental contamination [26]. Viability of EHV-1 in the environment could not be determined via qPCR and represents one limitation of the study. However, previous work has shown that EHV-1 remains viable on various environmental material (leather, cotton fabric, bedding, plastic) and in water for up to 48 hours and 3 weeks, respectively [27,28]. Concerning the two EHV-4 qPCR-positive stalls, there was no geographic association since the two stalls were from 2 different barns. High frequency of detection of the 2 gamma herpesviruses in nasal secretions account for the high association between qPCR-positive nasal secretions and the environment. While these 2 commensal respiratory viruses are unlikely to cause clinical disease, their detection in the environment is supporting environmental build up over time. The higher detection rate of *S. zooepidemicus* in stall sponges reflect environmental contamination as the streptococcal microorganisms are present not only in nasal secretions but also in feces [13]. In a recent study looking at respiratory pathogens in fecal material from 97 healthy horses at a multiday horse event, 55% of show horses tested qPCR-positive for *S. zooepidemicus*, which is comparable to the 40.9% of stall sponges testing positive in our study [13]. Study limitations related to the nature of the convenience study based on the voluntary enrollment of the participants. A random enrollment was not possible as written owner's consent was requested. However, based on the large number of show horses enrolled, the authors strongly believe that the data is truly representative of the distribution of the various respiratory pathogens. Further, the collection of daily nasal swabs from the same horses during the 5-day show event would likely have yielded a higher detection frequency of respiratory pathogens. Further, while the hunter/jumper event was the first show of the 2022 season at the study site, no environmental sponges were collected prior of stabling the horses. It is possible, although very unlikely, that some of the pathogens detected represent residual nucleic acids from previous show events. Viruses such as influenza virus and SARS-CoV-2 have been shown to remain detectable on contaminated surfaces for up to 7 weeks [29,30]. According to the organizer of the hunter/jumper show in California, the stalls were all cleaned and disinfected prior to the event.

In conclusion, direct and indirect monitoring for equine respiratory pathogens in horses attending multiday show events can be performed by testing nasal swabs and environmental samples using qPCR. While the qPCR testing of individual nasal swabs for respiratory viruses and bacteria gives an insight into active shedding, environmental stall swabs reflect past and present shedding and represent a more accurate measure of pathogen buildup over time. Pathogen spread via silent shedders or contaminated environment are essential factors for the development of outbreaks. The cluster of EHV-1 qPCR-positive stalls highlights the environmental buildup of respiratory virus and the risk for susceptible horses to potentially develop clinical disease if exposed. While the collection and testing of nasal and stall samples allows monitoring show horses and their direct environment, it is not a protocol meant to replace routine biosecurity measures. Future studies are needed in order to establish strategies aimed at reducing the risk of disease spread and to mitigate environmental contamination in order to reduce risk of exposure.

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References

[1] Traub-Dargatz JL, Pelzel-McCluskey AM, Creekmore LH, Geiser-Novotny S, Kasari TR, Wiedenheft AM, Bush EJ, Bjork KE. Case-control study of a multistate equine herpesvirus myeloencephalopathy outbreak. J Vet Intern Med 2013;27:339–46.
[2] Lesté-Lasserre C. Deadly viral outbreak ravages European horses. Science 2021;371:1297.
[3] Henninger RW, Reed SM, Saville WJ, Allen GA, Hass GF, Kohn CW, Sofaly C. Outbreak of neurologic disease caused by equine herpesvirus-1 at a university equestrian center. J Vet Intern Med 2007;21:157–65.
[4] Khusro A, Aarti C, Rivas-Caceres RR, Barbabosa-Pliego A. Equine herpesvirus-I infection in horses: recent updates on its pathogenicity, vaccination, and preventive management strategies. J Equine Vet Sci 2020;87:102923.
[5] Pusterla N, Kass PH, Mapes S, Johnson C, Barnett DC, Vaala W, Gutierrez C, McDaniel R, Whitehead B, Manning J. Surveillance programme for important equine infectious respiratory pathogens in the USA. Vet Rec 2011;169:12.
[6] Diaz-Mendez A, Viel L, Hewson J, Doig P, Carman S, Chambers T, Tiwari A, Dewey C. Surveillance of equine respiratory viruses in Ontario. Can J Vet Res 2010;74:271–8.
[7] McBrearty KA, Murray A, Dunowska M. A survey of respiratory viruses in New Zealand horses. N Z Vet J 2013;61:254–61.
[8] Doubli-Bounoua N, Richard EA, Léon A, Pitel PH, Pronost S, Fortier G. Multiple molecular detection of respiratory viruses and associated signs of airway inflammation in racehorses. Virol J 2016;13:197.
[9] Stasiak K, Dunowska M, Rola J. Prevalence and sequence analysis of equid herpesviruses from the respiratory tract of Polish horses. Virol J 2018;15:106.
[10] Smith FL, Watson JL, Spier SJ, Kilcoyne I, Mapes S, Sonder C, Pusterla N. Frequency of shedding of respiratory pathogens in horses recently imported to the United States. J Vet Intern Med 2018;32:1436–41.
[11] Pusterla N, Rice M, Henry T, Barnum S, James K. Investigation of the shedding of selected respiratory pathogens in healthy horses presented for routine dental care. J Vet Dent 2020;37:88–93.
[12] Couetil L, Ivester K, Barnum S, Pusterla N. Equine respiratory viruses, airway inflammation and performance in thoroughbred racehorses. Vet Microbiol 2021;257:109070.
[13] Stout AE, Hofmar-Glennon HG, André NM, Goodman LB, Anderson RR, Mitchell PK, Thompson BS, Lejeune M, Whittaker GR, Goodrich EL. Infectious disease surveillance of apparently healthy horses at a multi-day show using a novel nanoscale real-time PCR panel. J Vet Diagn Invest 2021;33:80–6.
[14] Pusterla N, Mapes S, Wademan C, White A, Hodzic E. Investigation of the role of lesser characterised respiratory viruses associated with upper respiratory tract infections in horses. Vet Rec 2013;172:315.
[15] Bernardino P, James K, Barnum S, Corbin R, Wademan C, Pusterla N. What have we learned from 7 years of equine rhinitis B virus qPCR testing in nasal secretions from horses with respiratory signs. Vet Rec 2021;188:e26.
[16] Gildea S, Arkins S, Cullinane A. Management and environmental factors involved in equine influenza outbreaks in Ireland 2007-2010. Equine Vet J 2011;43:608–17.
[17] Newton JR, Wood JL, Dunn KA, DeBrauwere MN, Chanter N. Naturally occurring persistent and asymptomatic infection of the guttural pouches of horses with *Streptococcus equi*. Vet Rec 1997;140:84–90.
[18] Pringle J, Venner M, Tscheschlok L, Bächi L, Riihimäki M. Long term silent carriers of *Streptococcus equi* ssp. *equi* following strangles; carrier detection related to sampling site of collection and culture versus qPCR. Vet J 2019;246:66–70.
[19] Horsington J, Lynch SE, Gilkerson JR, Studdert MJ, Hartley CA. Equine picornaviruses: well known but poorly understood. Vet Microbiol 2013;167:78–85.
[20] Hartley CA, Dynon KJ, Mekuria ZH, El-Hage CM, Holloway SA, Gilkerson JR. Equine gammaherpesviruses: perfect parasites? Vet Microbiol 2013;167:86–92.
[21] Back H, Ullman K, Treiberg Berndtsson L, Riihimäki M, Penell J, Ståhl K, Valarcher JF, Pringle J. Viral load of equine herpesviruses 2 and 5 in nasal swabs of actively racing Standardbred trotters: Temporal relationship of shedding to clinical findings and poor performance. Vet Microbiol 2015;179:142–8.
[22] El-Hage CM, Mekuria ZM, Hartley CA, Gilkerson JR. Survey of equine herpesvirus-1, -2, -4 and -5 in 407 horses with and without respiratory disease. J Equine Vet Sci 2016;39:68–9.
[23] Negussie H, Gizaw D, Tesfaw L, Li Y, Oguma K, Sentsui H, Tessema TS, Nauwynck HJ. 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;64:1970–8.
[24] Newton JR, Laxton R, Wood JL, Chanter N. Molecular epidemiology of *Streptococcus zooepidemicus* infection in naturally occurring equine respiratory disease. Vet J 2008;175:338–45.
[25] Pusterla N, Wilson WD, Mapes S, Finno C, Isbell D, Arthur RM, Ferraro GL. Characterization of viral loads, strain and state of equine herpesvirus-1 using real-time PCR in horses following natural exposure at a racetrack in California. Vet J 2009;179:230–9.
[26] Price D, Barnum S, Mize J, Pusterla N. Investigation of the use of non-invasive samples for the molecular detection of EHV-1 in horses with and without clinical infection. Pathogens 2022;11:574.
[27] Dayaram A, Seeber PA, Greenwood AD. Environmental detection and potential transmission of equine herpesviruses. Pathogens 2021;10:423.
[28] Saklou NT, Burgess BA, Ashton LV, Morley PS, Goehring LS. Environmental persistence of equid herpesvirus type-1. Equine Vet J 2021;53:349–55.

[29] Thompson KA, Bennett AM. Persistence of influenza on surfaces. J Hosp Infect 2017;95:194–9.
[30] Mohan SV, Hemalatha M, Kopperi H, Ranjith I, Kumar AK. SARS-CoV-2 in environmental perspective: Occurrence, persistence, surveillance, inactivation and challenges. Chem Eng J 2021;405:126893.