

Short Communication

Prevalence of equine coronavirus in nasal secretions from horses with fever and upper respiratory tract infection

N. Pusterla, N. Holzenkaempfer, S. Mapes, P. Kass

EQUINE coronavirus (ECoV) has recently been associated clinically and epidemiologically with emerging outbreaks of pyrogenic and enteric disease in adult horses in Japan and the USA (Oue and others 2011, 2013, Pusterla and others 2013). Due to the inconsistent development of enteric signs in horses infected with ECoV and in comparison with the closely related pneumoenteric bovine coronavirus (BCoV), one can only wonder if this virus has a tropism to non-enteric epithelial cells, such as

the respiratory epithelium. Recently, a French group screened 395 faeces and 200 respiratory specimens submitted to a veterinary diagnostic laboratory for the presence of ECoV (Miszczak and others 2014). The samples had been collected from foals and adult horses suffering from mild respiratory or enteric disease. In that study, the researchers found ECoV in a total of 12 samples (11 faecal samples and one respiratory specimen). The ECoV quantitative PCR (qPCR)-positive respiratory sample had been collected from a nine-month-old foal suffering from respiratory infection. Therefore, the objective of the present study was to investigate the presence of ECoV in nasal secretions from horses with signs of fever and/or acute onset of upper respiratory tract infection submitted to a molecular diagnostic laboratory in the USA for the detection of common infectious respiratory pathogens.

The study population consisted of 2437 equids with acute onset of fever ($T > 101.5^{\circ}\text{F}$) and/or upper respiratory tract infection (depression, lethargy, nasal discharge, coughing) for which nasal secretions were submitted to the Real-time PCR Research and Diagnostics Core Facility, School of Veterinary Medicine, University of California at Davis for the detection of common respiratory pathogens (equine influenza virus (EIV), equine herpesvirus type 1/equine herpesvirus type 4 (EHV-1/EHV-4), and equine rhinitis A virus and equine rhinitis B virus (ERAV/ERBV), *Streptococcus equi* subspecies *equi*) by qPCR (Pusterla and others 2011). Further, 187 healthy adult horses screened for respiratory pathogens for export purpose were included in this study as healthy controls. This horse population was used to determine if healthy adult horse could have detectable ECoV by qPCR in their nasal secretions. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California, Davis.

For each of the selected 2437 study cases and 187 healthy controls, previously processed and stored nucleic acid was used. Nucleic acid from nasal secretions was assayed for the presence of ECoV, as previously reported (Pusterla and others 2013).

Categorical analyses were performed using either the Fisher's exact or the Kruskal-Wallis test to determine the association between observations (age, breed, sex, clinical signs, qPCR status for common respiratory pathogens). ECoV PCR-positive horses

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TABLE 1: Month and year of presentation, signalment, use, clinical signs and qPCR results for common infectious respiratory pathogens in 17 ECoV qPCR-positive horses

Horse number	Month/year	Signalment (age (years)/breed/sex)	Use	Clinical signs	qPCR results for respiratory pathogens
1	1/2013	3/TB/stallion	Racing	102.5°F	EHV-4
2	11/2013	8/Arabian/gelding	Unknown	Depression, nasal discharge, coughing, 100.8°F	EHV-4
3	1/2014	10/TWH/mare	Pleasure	Not reported	<i>Streptococcus equi</i> subspecies <i>equi</i>
4	2/2014	8/QH/gelding	Unknown	Depression, anorexia, nasal discharge, coughing, 104.0°F	EIV
5	2/2014	14/APH/gelding	Show	Depression, anorexia, nasal discharge, 101.0°F	Negative
6	2/2014	10/APH/gelding	Pleasure	Depression, anorexia, nasal discharge, coughing, 102.5°F	<i>S equi</i> subspecies <i>equi</i>
7	3/2014	5/unknown/unknown	Unknown	Depression, anorexia, coughing, 102.8°F	Negative
8	3/2014	8/QH/gelding	Unknown	Not reported	Negative
9	3/2014	1.5/QH/filly	Unknown	Nasal discharge, coughing, 101.6°F	<i>S equi</i> subspecies <i>equi</i> /ERBV
10	4/2014	1/QH/gelding	Pleasure	Nasal discharge, 101.1°F	Negative
11	4/2014	1/QH/gelding	Pleasure	104.7°F	ERBV
12	5/2014	2/Appaloosa/gelding	Unknown	Anorexia, 102.0°F	EHV-4
13	5/2014	18/unknown/unknown	Unknown	Depression, anorexia, coughing, 103.5°F	Negative
14	5/2014	7/QH/gelding	Show	Depression, anorexia, nasal discharge, 104.4°F	Negative
15	5/2014	9/unknown/mare	Unknown	Nasal discharge, coughing, 101.1°F	Negative
16	5/2014	7/WB/gelding	Show	Depression, anorexia, coughing, 99.0°F	Negative
17	5/2014	6/TB/mare	Breeding	Not reported	ERBV

APH, American Paint Horse; ECoV, equine coronavirus; EHV-4, equine herpesvirus type 4; EIV, equine influenza virus; ERBV, equine rhinitis B virus; QH, Quarter Horse; qPCR, quantitative PCR; TB, Thoroughbred; TWH, Tennessee Walking Horse; WB, Warmblood.

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TABLE 2: Signalment, use and clinical signs in 2437 equids with fever and acute onset of upper respiratory tract infection tested for ECoV in nasal secretions from January 2013 to December 2014

	ECoV PCR-negative equids (2420)	ECoV PCR-positive equids (17)
Age (years)		
<1	209 (8.6%)	0
1-5	677 (28.0%)	6 (35.3%)
6-10	540 (22.3%)	9 (52.9%)
11-15	384 (15.9%)	1 (5.9%)
16-20	150 (6.2%)	1 (5.9%)
>20	124 (5.1%)	0
Not reported	336 (13.9%)	0
Breed		
Thoroughbred	312 (12.9%)	2 (11.8%)
Quarter Horse	738 (30.5%)	6 (35.3%)
Warmblood	301 (12.4%)	1 (5.9%)
American Paint	114 (4.7%)	2 (11.8%)
Pony	125 (5.2%)	0
Arabian	51 (2.1%)	1 (5.9%)
Standardbred	47 (1.9%)	0
Spanish	30 (1.2%)	0
Draft	74 (3.1%)	0
Mule/donkey	8 (0.3)	0
Others	195 (8.1%)	2 (11.8%)
Not reported	425 (17.6)	3 (17.5%)
Gender		
Mare/filly	829 (34.3%)	4 (23.5%)
Gelding	930 (38.4%)	10 (58.8%)
Stallion/colt	167 (6.9%)	1 (5.9%)
Not reported	494 (20.4%)	2 (11.8%)
Use		
Show	523 (21.6%)	3 (17.6%)
Pleasure	525 (21.7%)	4 (23.5%)
Racing	135 (5.6%)	1 (5.9%)
Breeding	128 (5.3%)	1 (5.9%)
Others	169 (7.0%)	0
Not reported	940 (38.8%)	8 (47.1%)
Clinical signs		
Depression yes	1314 (54.3%)	8 (47.1%)
Depression no	762 (31.5%)	6 (35.3%)
Depression unknown	344 (14.2%)	3 (17.6%)
Anorexia yes	1048 (43.3%)	8 (47.1%)
Anorexia no	1044 (43.1%)	6 (35.3%)
Anorexia unknown	328 (13.6%)	3 (17.6%)
Fever yes	1344 (55.5%)	9 (52.9%)
Fever no	575 (23.8%)	5 (29.4%)
Fever unknown	501 (20.7%)	3 (17.6%)
Nasal discharge yes	1609 (66.5%)	8 (47.1%)
Nasal discharge no	548 (22.6%)	6 (35.3%)
Nasal discharge unknown	263 (10.9%)	3 (17.6%)
Coughing yes	1104 (45.6%)	8 (47.1%)
Coughing no	968 (40.0%)	6 (35.3%)
Coughing unknown	348 (14.4%)	3 (17.6%)
qPCR results		
EHV-1 PCR-positive	42 (1.7%)	0
EHV-1 PCR-negative	2378 (98.3%)	17 (100%)
EHV-4 PCR-positive	207 (8.6%)	3 (17.6%)
EHV-4 PCR-negative	2213 (91.4%)	14 (82.4%)
EIV PCR-positive	223 (9.2%)	1 (5.9%)
EIV PCR-negative	2197 (90.8%)	16 (94.1%)
ERAV/ERBV PCR-positive	70 (2.9%)	3 (17.6%)
ERAV/ERBV PCR-negative	2350 (97.1%)	14 (82.4%)
<i>Streptococcus equi</i> subspecies <i>equi</i> PCR-positive	131 (5.4%)	3 (17.6%)
<i>S equi</i> subspecies <i>equi</i> PCR-negative	2289 (94.6%)	14 (82.4%)

ECoV, equine coronavirus; EHV-1, equine herpesvirus type 1; EHV-4, equine herpesvirus type 4; EIV, equine influenza virus; ERAV, equine rhinitis A virus; ERBV, equine rhinitis B virus.

were compared with the ECoV PCR-negative index cases. For all statistical analyses, the values of $P \leq 0.05$ were considered significant.

A total of 17/2437 (0.7 per cent) index cases tested qPCR-positive for ECoV (Table 1). All the healthy control horses tested

qPCR-negative for ECoV. ECoV qPCR-positive horses were detected only during the winter and spring months. The age of the ECoV qPCR-positive horses ranged from 1 to 18 years (median 7 years; Table 2), while the age of the ECoV qPCR-negative horses ranged from two weeks to 36 years (median 7 years). Age was not available for 336 of the ECoV qPCR-negative equids. The breeds of ECoV qPCR-positive horses included Quarter Horse (6), Thoroughbred (2), American Paint Horse (2), Warmblood (1), Arabian (1) and other breeds (2), while the breed was not reported for three horses. Among the 17 ECoV qPCR-positive cases, there were four mares, one stallion, 10 geldings and two animals with no reported sex. The actual or intended use for the ECoV qPCR-positive animals was pleasure riding (4), show (3), racing (1) and breeding (1). For eight ECoV qPCR-positive horses, the use was not reported. Fever (52.9 per cent), depression (47.1 per cent), anorexia (47.1 per cent), nasal discharge (47.1 per cent) and coughing (47.1 per cent) were the most commonly reported clinical signs among ECoV qPCR-positive animals. Clinical signs were not available for three ECoV qPCR-positive horses.

qPCR testing for common infectious respiratory pathogens revealed that among the 17 ECoV qPCR-positive horses, nine animals tested positive for concurrent pathogens, including EHV-4 (3), ERAV/ERBV (3), *S equi* subspecies *equi* (3) and EIV (1). Among the ECoV qPCR-negative horses, 223 (9.2 per cent) tested PCR positive for EIV, 207 (8.6 per cent) for EHV-4, 131 (5.4 per cent) for *S equi* subspecies *equi*, 70 (2.9 per cent) for ERBV and 42 (1.7 per cent) for EHV-1.

No significant associations were found between ECoV qPCR-positive horses and ECoV qPCR-negative index cases for age, breed, sex, use, specific clinical signs and qPCR positivity for EHV-4, EHV-1, EIV and *S equi* subspecies *equi* ($P < 0.05$). A higher-than-expected prevalence of ECoV qPCR-positive animals with concurrent detection of ERBV was found ($P = 0.01$) when compared with the ECoV qPCR-negative index cases that tested qPCR-positive for ERBV.

The results of the present study showed that ECoV is detected by qPCR in a small percentage of horses with acute onset of fever and/or upper respiratory tract infection, while EHV-1, EHV-4, EIV, ERAV/ERBV and *S equi* subspecies *equi* remain the predominant respiratory pathogens associated with upper respiratory tract infection. It is of interest to notice that ECoV qPCR-positive horses were only detected during the winter and spring months, which is in agreement with the detection of BCoV in nasal secretions of calves with bovine respiratory disease (O'Neill and others 2014). Further, the majority of the ECoV outbreaks reported in the literature occurred during the cold months of the year (Oue and others 2013, Pusterla and others 2013). This may relate to husbandry practices and greater population of healthy foals known to shed ECoV in their faeces, therefore contributing to environmental contamination.

As with the closely related BCoV, ECoV has recently been detected in the nasal secretions of 1/200 foals and adult horses suffering from respiratory disease (Miszczak and others 2014) as well as the nasal secretions from three nine-month-old to 10-month-old Japanese draft foals experimentally infected with ECoV (Nemoto and others 2014). Despite a small number of ECoV qPCR-positive study horses, there was no difference in frequency of the predominant clinical signs between horses with monoinfection of ECoV and horses with coinfection of ECoV and common infectious respiratory pathogens. The lack of reported respiratory signs associated with ECoV outbreaks and experimental infection may relate to the infrequent nature of such signs or the lack of tropism of ECoV to the respiratory epithelium. These findings are also supported by the observation that none of the healthy adult horses tested qPCR-positive for ECoV in their nasal secretions.

BCoV is considered a pneumoenteric virus, causing not only enteric disease but also mild upper respiratory signs (Saif and others 1986, Tsunemitsu and others 1999, Trávén and others 2001). BCoV has been shown in one recent study (O'Neill and

others 2014) to be the most frequently detected partner virus found in combination with other respiratory viruses in nasal secretions from calves associated with respiratory disease outbreaks. Out of the 17 ECoV qPCR-positive horses, nine (52.9 per cent) showed coinfection with either EHV-4, EIV, ERBV or *S equi* subspecies *equi*. To the authors' knowledge, this is the first time coinfection of ECoV with common infectious respiratory tract pathogens has been detected via qPCR in nasal secretions of horses with fever and/or upper respiratory tract infection. While the pathogenic role of common equine infectious respiratory tract pathogens is well established, the role of ECoV as a mono-infection or coinfection in the upper respiratory tract still needs to be determined. One can only speculate that, similar to BCoV and its association with bovine respiratory disease, concurrent infections with two or more viruses can lead to a synergistic pathological effect (Srikumaran and others 2007).

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