# Frequency of molecular detection of equine coronavirus in faeces and nasal secretions in 277 horses with acute onset of fever

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## Abstract

**Context** Due to the inconsistent development of enteric signs associated with ECoV infection in adult horses, many practitioners collect nasal secretions rather than feces for the molecular diagnostic work-up of such horses.

**Main conclusion** ECoV infection should be considered in horses presenting with acute onset of fever, especially when nasal discharge is absent as one of the cardinal clinical sign.

**Approach** A total of 277 adult horses with acute onset of fever were enrolled in this study. Feces were tested for ECoV and nasal secretions for common respiratory pathogens (equine herpesvirus (EHV)-1, EHV-4, equine influenza virus (EIV), equine rhinitis viruses (ERVs) and *Streptococcus equi ss. equi*) and ECoV by qPCR. Each submission was accompanied by a questionnaire requesting information pertaining to signalment, use, recent transportation, number of affected horses on the premise and presence of clinical signs at the time of sample collection.

**Results** The total number of horses testing qPCR-positive for ECoV in feces was 20 (7.2%), 4 of which also tested qPCR-positive for ECoV in nasal secretions. In the same population 9.0% of horses tested qPCR-positive for EHV-4, 6.1% for EIV, 4.3% for *Streptococcus equi ss. equi*, 3.2% for ERVs and 0.7% for EHV-1. Draft horses, pleasure use, multiple horses affected on a premise and lack of nasal discharge were significantly associated with ECoV qPCR-positive horses.

**Interpretation** The present study results showed that 7.2% of horses with acute onset of fever tested qPCR-positive for ECoV in feces, highlighting the importance of testing such horses for ECoV in feces. The various prevalence factors associated with ECoV qPCR-positive status likely relate to the high infectious nature of ECoV and breed-specific differences in management and husbandry practices.

**Significance of findings** ECoV infection should be suspected and tested for in horses presenting with acute onset of fever, lethargy and anorexia with no respiratory signs. A two-step approach should be consider in which respiratory secretions and feces should be collected from such horses and submitted to a diagnostic laboratory. If the respiratory secretions test negative by qPCR for a panel of respiratory pathogens, feces already submitted to the laboratory should be tested for ECoV.


## Introduction

Equine coronavirus (ECoV) is considered an enteric virus of foals and has only recently been associated with emerging infections in adult horses in Japan, USA and Europe.[1–4] Clinical signs most commonly seen in adult horses infected with ECoV include anorexia, lethargy and fever, and less frequently diarrhoea, colic and neurological deficits.[1–3] The morbidity rate during outbreaks has been reported to range from 20 per cent to 83 per cent, while the mortality rate due to endotoxaemic shock, septicaemia or hyperammonaemia-associated encephalopathy can reach up to 10 per cent.[5] A clinical diagnosis of ECoV infection is generally supported by haematological changes (neutropenia and/or lymphopenia), the exclusion of other infectious agents and the detection of ECoV in faeces via quantitative

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**Table 1** Signalment, use, transportation history, number of affected horses and clinical signs associated with ECoV qPCR status in 277 horses

| | ECoV faecal sample negative by qPCR (n=257) | ECoV faecal sample positive by qPCR (n=20) |
|---|---|---|
| Age (years) | | |
| Less than 1 (n=18) | 18 (7%) | 0 (0%) |
| 1–5 (n=57) | 50 (19%) | 7 (35%) |
| 6–10 (n=58) | 57 (22%) | 1 (5%) |
| 11–15 (n=63) | 58 (22%) | 5 (25%) |
| 16–20 (n=41) | 37 (14%) | 4 (20%) |
| Over 20 (n=27) | 25 (10%) | 2 (10%) |
| Unknown (n=13) | 12 (5%) | 1 (5%) |
| Breed | | |
| Quarter horse (n=78) | 73 (28%) | 5 (25%) |
| Warmblood (n=42) | 40 (16%) | 2 (10%) |
| Thoroughbred (n=21) | 20 (8%) | 1 (5%) |
| Paint (n=15) | 15 (6%) | 0 (0%) |
| Arabian (n=14) | 14 (5%) | 0 (0%) |
| Draft horse (n=14) | 10 (4%) | 4 (20%)* |
| Pony/miniature (n=21) | 19 (7%) | 2 (10%) |
| Other breed (n=55) | 51 (19%) | 4 (20%) |
| Unknown (n=17) | 15 (6%) | 2 (10%) |
| Sex | | |
| Female (n=89) | 79 (31%) | 10 (50%) |
| Male† (n=174) | 166 (64%) | 8 (40%) |
| Unknown (n=14) | 12 (5%) | 2 (10%) |
| Use | | |
| Competition (n=92) | 90 (35%) | 2 (10%) |
| Pleasure horse (n=138) | 124 (48%) | 14 (70%)* |
| Breeding (n=11) | 11 (4%) | 0 (0%) |
| Other use (n=4) | 3 (1%) | 1 (5%) |
| Unknown (n=32) | 29 (11%) | 3 (15%) |
| History of transport | | |
| Yes (n=60) | 58 (23%) | 2 (10%) |
| No (n=189) | 172 (66%) | 17 (85%) |
| Unknown (n=28) | 27 (11%) | 1 (5%) |
| Affected horses on property | | |
| Single (n=147) | 140 (61%) | 7 (37%) |
| Multiple (n=100) | 88 (34%) | 12 (60%)* |
| Unknown (n=30) | 29 (11%) | 1 (5%) |
| Clinical signs | | |
| Nasal discharge (n=147) | 142 (55%) | 5 (25%)* |
| No nasal discharge (n=130) | 115 (45%) | 15 (75%)* |
| Cough (n=85) | 81 (32%) | 4 (20%) |
| No Cough (n=192) | 176 (68%) | 16 (80%) |
| Rectal temperature (°C)‡ | 39.4 | 39.9* |
| Anorexia (n=206) | 187 (73%) | 19 (95%) |
| No anorexia (n=71) | 70 (27%) | 1 (5%) |
| Lethargy (n=230) | 212 (82%) | 18 (90) |
| No lethargy(n=47) | 47 (18%) | 0 (0%) |
| qPCR results of nasal secretions | | |
| Positive for EHV-1 (n=2) | 1 (<1%) | 1 (5%) |
| Positive for EHV-4 (n=25) | 24 (9%) | 1 (5%) |
| Positive for EIV (n=71) | 17 (6%) | 0 (0%) |
| Positive for *S equi* (n=12) | 12 (5%) | 0 (0%) |
| Positive for ERVs (n=9) | 9 (3.5%) | 0 (0%) |

*Denotes statistically significant differences between groups.
†'Male' includes geldings and stallions.
‡Median.
ECoV, equine coronavirus; EHV, equine herpesvirus; EIV, equine influenza virus; ERV, equine rhinitis virus; qPCR, quantitative real-time PCR.

real-time PCR (qPCR).[6] However, due to the inconsistent development of enteric signs associated with ECoV infection, many practitioners collect nasal secretions rather than faeces for the diagnostic workup of such horses. In comparison to the closely related bovine coronavirus, a pneumoenteric virus associated with enteric and respiratory signs, ECoV is seldom detected in respiratory secretions of healthy and sick horses.[4 7 8] Therefore, the objective of this study was to determine the frequency of detection of ECoV in both, nasal secretions and faeces from horses presented to equine practitioners with fever and at least one or more of the following clinical signs: lethargy, anorexia, nasal discharge and cough.

## Materials and methods

Case definition included horses with acute onset of fever (T≥38.6°C) and at least one or more of the following clinical signs: lethargy, anorexia, nasal discharge and cough. Horses with colic and changes in faecal character as well as horses with a chronic disease history were excluded from this study. The horses were presented to equine primary care providers across USA from August 2016 to July 2017 (12 months). Nasal secretions were collected using two 6' rayon-tipped swabs (Puritan Products Company, Guilford, Maine, USA). Further, veterinarians were asked to collect one to two faecal balls in a faecal cup. Samples were kept refrigerated and shipped on ice overnight to the laboratory. Further, each submission was accompanied by a questionnaire requesting information pertaining to signalment (age, breed, sex), use (competition, pleasure use, breeding, others), transportation during the past 14 days, number of affected horses on the premise (single versus multiple cases) and presence of clinical signs at the time of sample collection (general attitude, appetite, rectal temperature, nasal discharge, presence of cough).

Nucleic acid extractions from nasal secretions and faeces were performed on the day of sample arrival to the laboratory using an automated nucleic acid extraction system (CAS-1820 X-tractor Gene, Corbett Life Science, Australia) according to the manufacturer's recommendations. Nasal secretions were assayed for a panel of respiratory pathogens including equine herpesvirus (EHV)-1, EHV-4, equine influenza virus (EIV), equine rhinitis A virus, equine rhinitis B virus, equine rhinitis viruses (ERVs) and *Streptococcus equi* subspecies *equi* (*S equi*).[9] Further, nasal secretions and faeces were tested for the molecular presence of ECoV as previously reported.[3]

Associations between horses either qPCR-positive or qPCR-negative for ECoV and categorical variables were evaluated using Fisher's exact test and exact $x^2$ test. Univariate logistic regression of each prevalence factor was performed to determine the prevalence odds ratios associated with age, breed, sex, use, transportation, number of affected horses and clinical signs. The relatively small number of ECoV-positive outcomes precluded the use of multivariate models to more fully explore the marginal associations between predictors and qPCR-positive status. All statistical evaluations analyses were performed in Stata V.14 (Stata statistical software, College Station, Texas, USA) and statistical significance was set at P<0.05.

**Table 2** Univariate logistic regression results for the association between ECoV qPCR status and selected predictors (signalment, use, transportation history, number of affected horses and clinical signs) in 20 horses

| | Odds ratios for ECoV faecal qPCR-positive status (n=20) |
|---|---|
| Breed | |
| Quarter horse | Reference |
| Draft horse | 5.84 (1.34, 25.4)* |
| Use | |
| Competition | Reference |
| Pleasure | 5.10 (1.13, 22.9)* |
| Affected horses on property | |
| Single | Reference |
| Multiple | 2.72 (1.03, 7.20)* |
| Clinical signs | |
| Nasal discharge | 0.26 (.09, 0.74)* |

*P value < 0.05.
ECoV, equine coronavirus; qPCR, quantitative real-time PCR.

## Results

Over a 12-month period, nasal secretions and faeces from 277 horses were provided to the laboratory for qPCR testing. A total of 20/277 (7.2 per cent) horses tested qPCR-positive for ECoV. All 20 horses tested qPCR-positive for ECoV in faeces (absolute genome equivalents from 6,543 to 1.2 x $10^5$ per g of feces) while 4 of these horses also tested qPCR-positive for ECoV in nasal secretions. The same study population tested qPCR-positive for EHV-4 in 25 (9.0 per cent) cases, for EIV in 17 (6.1 per cent) cases, for *S equi* in 12 (4.3 per cent) cases, for ERVs in 9 (3.2 per cent) cases and for EHV-1 in 2 (0.7 per cent) cases. There was no significant difference in comorbidity between ECoV qPCR status and the common equine respiratory pathogens (P<0.05, table 1).

Age, breed, sex, use, recent transportation, number of affected horses on the premise and clinical signs between the two ECoV qPCR groups are listed in table 1. In the qPCR-positive ECoV group there were significantly more draft horses, more horses used for pleasure riding and more horses originating from a premise with multiple horses affected (P<0.05) compared with horses from the ECoV qPCR-negative group. When clinical signs were compared between the two groups, the rectal temperature was significantly higher (P<0.05) in the ECoV qPCR-positive group (median 39.9°C) compared with the ECoV qPCR-negative group (median 39.4°C). Significantly fewer ECoV qPCR-positive horses displayed nasal discharge when compared with ECoV qPCR-negative horses (P<0.05). There were no statistically significant differences in age, sex, recent transportation, cough, lethargy and anorexia between the two ECoV qPCR groups (P>0.05).

In univariate models, draft horses, pleasure use and multiple horses affected from the same premise were more frequently infected with ECoV when compared with ECoV qPCR-negative horses (table 2). ECoV qPCR-negative horses had a higher odds ratio of presenting for nasal discharge when compared with ECoV qPCR-positive horses. Age, sex, recent transportation, cough, anorexia and lethargy were not risk factors associated with ECoV qPCR-positive status.

## Discussion

Most reports on natural and experimental ECoV infection in adult horses have listed fever, lethargy and anorexia as the three cardinal clinical signs.[1–4 10] While these clinical signs reflect a systemic inflammatory response, their interpretation makes it difficult for equine practitioners to determine their origin and to choose the appropriate diagnostic sample to investigate potential pathogens. Equine practitioners are more likely to collect nasal secretions rather than faeces because of the assumption that the clinical complaint is more likely to originate from an upper airway infection. The overall detection rate of equine infectious pathogens in both nasal secretions and faeces was 29 per cent. The frequency of detection of common respiratory pathogens was lower than previous reports using similar horse populations.[11 12] The reason for the observed discrepancy likely relates to case inclusion criteria. Previous studies focused on horses with acute onset of fever and at least one respiratory sign, making the syndrome more likely associated with the respiratory apparatus. While adult horses remain susceptible to infectious respiratory pathogens, the clinical expression of disease is often milder or remains subclinical especially in previously vaccinated horses.[13] This observation further highlights the diagnostic challenge equine practitioners face when presented with a horse displaying fever and other unspecific signs. The present study results showed that 7.2 per cent of horses with acute onset of fever tested qPCR-positive for ECoV in faeces, while only 1.4 per cent of them had detectable ECoV in nasal secretions by qPCR. It appears based on experimental studies that most ECoV qPCR-positive nasal results occur the day after peak faecal shedding.[10] This observation is suggestive of environmental contamination and secondary viral occupation of the nares as opposed to true haematogenous spread and colonisation of the nares. The inclusion of a healthy horse population lacking clinical signs of infection would have been helpful in determining the frequency of subclinical shedding of respiratory pathogens and ECoV. The detection of ECoV in faeces of healthy foals and adult horses has previously been shown to be uncommon.[8 14] Therefore, the molecular detection of ECoV in faeces from a horse with fever, lethargy and anorexia strongly supports the laboratory diagnosis of coronavirus infection.

Comorbidity between respiratory pathogens and ECoV was seldom observed in the present study population. A previous study performed on healthy foals and foals with diarrhoea from Central Kentucky reported similar frequencies of qPCR detection of ECoV in faeces.[15] The two groups, however, differed in the rate

of comorbidity, with foals with diarrhoea commonly showing comorbidity with other enteric pathogens in comparison to healthy foals. Unfortunately, no additional enteric pathogens of adult horses were investigated in the present study.

The study results showed no difference in age and sex distribution between the two ECoV qPCR groups. Among the breeds, draft horses showed a significantly higher ECoV qPCR-positive status compared with ECoV qPCR-negative horses. The first ECoV outbreaks reported from Japan occurred in young racing draft horses.[1 2] Further, a recent ECoV seroprevalence study performed on healthy adult horses from the USA showed that draft horses were more likely to test seropositive.[16] It remains to be determined if breed is directly linked to increased susceptibility to ECoV infection or if this observation relates to breed-specific differences in management and husbandry practices. Pleasure use associated with ECoV qPCR-positive status was determined to be an artefact of the distribution of uses by the most prominently affected breed, which was draft horse. One would expect competition horses to be at a higher risk of testing ECoV qPCR-positive in comparison to other uses, knowing that performance horses are often subjected to increased movement, a recognised risk factor for the transmission of contagious pathogens. Multiple horses affected on a premise was positively associated with ECoV qPCR-positive status. This relates to the infectious nature of ECoV and the reported high morbidity rates associated with outbreaks.[1–3 5] The clinical signs observed in the ECoV qPCR-negative and qPCR-positive horse groups were very similar with the exception of nasal discharge. Absence of nasal discharge was significantly associated with an ECoV qPCR-negative status. This highlights the importance of testing faeces for non-respiratory pathogens, such as ECoV, when a horse presents with acute onset of fever, lethargy and anorexia with no respiratory signs. Expanding the faecal analysis to additional enteric pathogens could have increased the spectrum of infectious aetiologies associated with fever.

In conclusion, ECoV infection should be considered in adult horses presenting with acute onset of fever, especially when nasal discharge is absent as one of the cardinal clinical signs. While the collection and testing of nasal secretions for the detection of respiratory pathogens often predominate the diagnostic workup of such horses, equine veterinarians are encouraged to also submit faeces for qPCR testing against ECoV. Since the detection of ECoV by qPCR in nasal secretions is an infrequent event, the testing for ECoV should be restricted to faeces.

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