

# Detection of equine coronavirus in horses in the United Kingdom

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## Introduction

Equine coronavirus (ECoV) is a *Betacoronavirus* that has been associated with disease in individual animals and groups of horses, and over the past seven years has been increasingly reported as a cause of disease outbreaks—primarily in adult horses in the USA and Japan.<sup>1–3</sup> The main clinical signs associated with infection in adult horses are anorexia, lethargy and fever, with diarrhoea and mild colic signs less frequently reported.<sup>2–3</sup> More serious complications associated with infection include septicaemia and encephalopathy associated with hyperammonaemia, however these complications are rare and most horses recover with supportive care.<sup>3–4</sup> Leucopenia with lymphopenia and/or neutropenia are the main haematological abnormalities reported in clinical adult cases.<sup>3</sup> The virus primarily infects the small intestinal mucosa causing enteritis, and a faeco-oral route of transmission is suspected.<sup>5–7</sup>

Clinically affected horses have been shown to shed virus in their faeces for up to 11 days and ECoV has also been detected in the faeces of small numbers of healthy adult horses on premises where disease outbreaks have occurred.<sup>3,4</sup> qPCR testing of faeces is a sensitive diagnostic method to confirm ECoV infection. One study determined 90 percent accuracy between clinical status and PCR detection of ECoV infection in disease outbreaks.<sup>3</sup> Peak faecal shedding has been shown to occur three to four days after the development of clinical signs and there have been a few cases of early ECoV infection that have tested qPCR negative; however, these horses tested positive

24–48 hours later and repeat testing of a single or pooled faecal sample at a later time point may be warranted in qPCR negative cases where there is a clinical suspicion of disease.<sup>7</sup> The stability of ECoV in the environment is currently unknown.

ECoV has also been associated with enteric infection in foals, however it commonly presents a coinfection with other infectious agents and in one study in the USA was frequently detected in the faeces of healthy foals.<sup>8–11</sup>

The virus has an international distribution and molecular detection of ECoV has been reported in horses from the USA and Japan with the first detection in Europe being reported in France in the winter season of 2011/2012.<sup>12</sup> The current study reports the results of faecal testing for the presence of ECoV in the UK.

## Materials and methods

Quantitative PCR testing for ECoV was introduced at our laboratory in March 2015, and between March 2015 and July 2017, a total of 381 faecal samples from two groups of adult horses, yearlings, foals and donkeys were tested for the presence of ECoV RNA. Group 1 consisted of 156 faecal samples for which the submitting veterinary surgeon had requested ECoV testing while the second group comprised 225 faecal samples submitted from horses with no clinical suspicion of ECoV infection (table 1). Faecal samples were processed on the day of submission and nucleic acid was extracted using an automated system based on the manufacturer's instructions (Qiagen QIAamp cadof Pathogen Mini Kit). The qPCR assay was based on the detection of a specific 142bp product of the N gene of ECoV and was carried out following methods previously described.<sup>3</sup> A segment of the ECoV N gene from one positive case was sequenced using standard procedures.<sup>3</sup>

## Results

ECoV RNA was detected in samples from four horses in group 1 (2.6 per cent of group 1 horses). No ECoV RNA was detected in samples from group 2 horses (table 1).

The first positive case was detected in December 2016 and was a 19-year-old cob that presented with clinical signs of inappetence, lethargy, fever and low-grade colic. The

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**Table 1** Results of faecal PCR testing for ECoV RNA. Group 1 – horses and donkeys for which the submitting veterinary surgeon requested ECoV testing, Group 2 – horses with no clinical suspicion of ECoV infection.

	Animals (n)	Positive (n)	Positive (%)
Group 1			
Young foal (<4 months)	38	0	0
Foal (4 months to 1 year)	5	0	0
Yearling (1–2 years)	13	3	23.1
Adult (>2 years)	38	1	2.6
Donkey	62	0	0
Group 2			
Young foal (<4 months)	77	0	0
Foal (4 months to 1 year)	7	0	0
Yearling (1–2 years)	34	0	0
Adult (>2 years)	107	0	0

main haematological abnormalities were leucopenia with neutropenia and lymphopenia. The horse was hospitalised for observation and made a full recovery within four days without administration of any treatment. Two in contact horses, one adult and one foal, showed no clinical signs of disease. Partial sequencing of the N gene from this case showed 99 percent homology with previously reported ECoV strains from the USA and Japan (ECoV-NC99 and ECoV-Tokachi09).

The other positive cases were detected in March 2017 and were three Thoroughbred yearlings from the same premises. Main presenting clinical signs included weight loss and lethargy and all were diagnosed with concurrent larval cyathostomiasis. Faeces from one yearling were also ELISA positive for *Clostridium perfringens* enterotoxin and *Clostridium difficile* toxins A and B. Faecal samples from six healthy in contact yearlings tested qPCR negative for ECoV RNA. All yearlings were given fenbendazole, ivermectin, metronidazole, omeprazole and prednisolone. Two were given intravenous infusions of colloid (hetastarch). Additionally, the most severely affected individual was also treated with low molecular weight heparin, aspirin, morphine, flunixin, paracetamol, pentoxifylline and total intravenous nutrition. Two yearlings were successfully discharged from the hospital after six days, while the third was hospitalised for 10 days. After discharge, the farm's worm control policy was reviewed and modified.

## Discussion

This is the first reported molecular detection of ECoV from horses in the UK. The first positive case was an adult horse and the clinical signs and haematological abnormalities in this case were similar to those reported in clinical cases of ECoV infection in other countries<sup>2 3</sup> and ECoV infection was considered to be the likely cause of disease in this case. The ECoV strain detected showed close homology with previously reported strains from the USA and Japan.

The significance of ECoV infection in the three younger horses was more difficult to determine and larval cyathostomiasis was considered to be the primary cause of the clinical signs; however, coronavirus may have played a role as a coinfection. In a study carried out in foals in central Kentucky, ECoV was detected in the faeces of a similar

percentage of healthy foals and foals with diarrhoea.<sup>11</sup> In foals with diarrhoea it was often present as a coinfection and this is similar to the three yearlings in the current study. It is also possible that detection of ECoV in these yearlings was an incidental finding, however our initial screening suggests that prevalence of ECoV infection in the UK is low in both healthy horses and horses with enteric disease, similar findings to a previous study carried out in Japan which showed the presence of ECoV in the faeces of few healthy foals and not in diarrhoeic foals.<sup>13</sup> The three yearlings were given supportive care and primarily treated for larval cyathostomiasis and associated complications. There is currently no specific treatment for ECoV infection and most adult horses recover spontaneously or with supportive care.<sup>7</sup>

Interestingly, in the USA, disease is more common during the colder months of October to March and this is similar to the positive cases detected in the UK.<sup>7</sup>

The true incidence of ECoV infection in horses in the UK is not known. Our initial study suggests prevalence is relatively low, however further studies are required to further investigate this finding. However, in horses with compatible clinical signs, ECoV should be considered as a potential pathogen with qPCR testing of faeces being the diagnostic test of choice.

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