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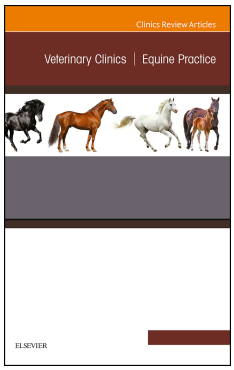

Equine Coronaviruses

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PII: S0749-0739(22)00072-4

DOI: https://doi.org/10.1016/j.cveq.2022.11.008

Reference: VEP 901

To appear in: *VETERINARY CLINICS OF NORTH AMERICA: EQUINE PRACTICE*

Please cite this article as: Pusterla N, Equine Coronaviruses, *VETERINARY CLINICS OF NORTH AMERICA: EQUINE PRACTICE* (2022), doi: https://doi.org/10.1016/j.cveq.2022.11.008.

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Synopsis: Coronaviruses are a group of related RNA viruses that cause diseases in mammals and birds. In equids, equine coronavirus has been associated with diarrhea in foals and lethargy, fever, anorexia and occasional gastrointestinal signs in adult horses. The challenge with ECoV infection is recognizing the entity based on clinical and hematological abnormalities and support a diagnosis via molecular detection of coronavirus in feces. There are presently no specific treatment or preventive measures for ECoV infection. While horses appear to be susceptible to the human SARS-CoV-2 based on the high homology to the ACE-2 receptor, they appear to be incidental hosts because of occasional SARS-CoV-2 spillover from humans. However, until more clinical and seroepidemiological data are available, it remains important to monitor equids for possible transmission from humans with clinical or asymptomatic COVID-19.

Key Words: Equine coronavirus (ECoV); Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2); Epidemiology; Pathogenesis; Clinical Signs; Diagnosis; Treatment and Prevention

Key Points

- Equids are susceptible to SARS-CoV-2 based on the high homology to the ACE-2 receptor; however, only silent infection has been documented at the present time.
- Horses are unlikely to contribute to the spread of SARS-CoV-2; however, with emerging coronavirus variants, it remains prudent to prevent interactions between COVID-19 patients and equids.
- ECoV infection in adult equids is often characterized by unspecific clinical signs such as fever, lethargy and anorexia, while changes in fecal character and colic are infrequently observed in infected animals.
- A diagnosis of ECoV should be considered when multiple adult horses are affected by fever, lethargy, anorexia with or without gastrointestinal signs and hematological changes (leucopenia due to neutropenia and/or lymphopenia) are consistent with an underlying viral disease. A laboratory diagnosis of ECoV is supported by the molecular detection of ECoV in feces.
- ECoV infection is often self-limiting requiring at best supportive treatment with NSAIDs and polyionic fluids and antimicrobials if endotoxemia/septicemia is suspected.
- The prevention of ECoV infection should focus on the implementation of routine management practices aimed at reducing the likelihood of introducing and disseminating ECoV at any horse-based premise as well as the timely isolation of horses with suspected ECoV infection.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Although successful experimental transmission of SARS-CoV-2 has been documented in a variety of domestic and laboratory animal species, including dogs, cats, ferrets, pigs, New Zealand white rabbits, mice and Syrian hamsters,[1-5] suspected natural transmission through the spillover from infected humans to domestic animals has only been reported in dogs and cats.[6-11] Susceptibility and exposure to SARS-CoV-2 are the two main prerequisites for any domestic animal to develop clinical or subclinical infection.[12] Little work has been focusing on characterizing the role of equids in the COVID-19 pandemic. Susceptibility of equids (horses and donkeys) has been established by comparative analysis of ACE-2 protein sequences and the data showed that equids have low affinity to bind.[13] A recent functional and genetic study determined that viral receptor ACE-2 orthologs from 44 different species, including horses, were able to bind SARS-CoV-2 spike protein, therefore supporting viral entry.[14]

Experimental intranasal infection with an ancestral SARS-CoV-2 strain (virus strain 2019-nCoV/USA-WA1/2020) in a single horse has been reported in the literature.[15] The attempt to experimentally infect the single horse failed, which is not surprising, considering that experimental infections of equids using the closely-related Middle East respiratory syndrome coronavirus (MERS-CoV) showed lack of immune response and no viral RNA detected in respiratory secretions.[16] However, one must keep in mind that SARS-CoV-2 has continued to evolve and adapt, meaning that, it is possible that more contemporary human-adapted variants will show greater potential to replicate in equids.

Molecular detection of SARS-CoV-2 in nasal secretions and/or feces of healthy horses and horses with acute onset of fever and respiratory signs has remained unsuccessful.[17,18] Even horses with close contact to SARS-CoV-2 infected keepers, track

workers and riders have no to little evidence of spillover infection.[18,19] While molecular detection of SARS-CoV-2 in respiratory secretions is limited to a short shedding period, especially in silent shedders, serology is more sensitive at capturing past-exposure. A recent serological survey from China, did not find antibodies specific to SARS-CoV-2 from healthy horses.[20] This was in sharp contrast to a study evaluating silent transmission of SARS-CoV-2 between racetrack workers with asymptomatic COVID-19 and racing Thoroughbred horses.[18] This study detected specific antibodies to SARS-CoV-2 in 35/587 (5.9%) racing horses. Current CDC guidelines recommend that owners with SARS-CoV-2 avoid any close contact with their animals, including equids. A recent study documented seroconversion in one of two horses with direct contact to an individual (horse owner) with clinical COVID-19.[19] None of the two horses had detectable SARS-CoV-2 in nasal secretions, blood and feces.

While horses are apparently susceptible to SARS-CoV-2 and are likely to become infected through spillover from COVID-19 individuals, horses are unlikely to contribute to the spread of SARS-CoV-2. However, it is important to continue to monitor possible human-to-horse transmission, especially with the emergence of highly transmissible SARS-CoV-2 variants,[21] and to recommend that COVID-19 individuals avoid close contact with companion animals (dogs, cats, ferrets, horses).

Equine coronavirus (ECoV)

Etiology

Coronaviruses are single-stranded, positive-sense, non-segmented, enveloped RNA viruses responsible for organ-specific syndromes in a variety of mammalian and avian species.[22] The family *Coronaviridae* is subdivided in two subfamilies, *Torovirinae*

and *Coronavirinae*, with the latter subfamily containing four genera defined on the basis of serological cross-reactivity and genetic differences: *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus*.[23] The *Betacoronavirus* genera is further divided into five subgenera (*Sarbecovirus*, *Embecovirus*, *Merbecovirus*, *Nobecovirus*, and *Hibecovirus*).[24] ECoV belongs to the *Embecovirus* subgenera and is phylogenetically related to bovine coronavirus (BCoV), human OC43 and HKU1 coronaviruses, canine respiratory coronavirus, mouse hepatitis virus and sialodacryoadenitis virus coronavirus OC43, and porcine hemagglutinating encephalomyelitis virus. SARS-CoV-2 belongs to the *Sarbecovirus* subgenera, while Middle East respiratory syndrome coronavirus (MERS-CoV) belongs to the *Merbecovirus* subgenera.[24]

Partial and complete genome sequences from a small number of ECoV isolates from Japan, the USA and Europe has shown high level of sequence homology ranging between 97.2 and 99.6%.[25-31] One recent report identified a novel ECoV variant from the small intestinal samples collected from 2 donkey foals with diarrhea.[32] Bioinformatic analyses showed that the novel ECoV variant shared the highest sequence identity of 97.05% with the first identified ECoV strain - NC99.[25,32] The genetic database will likely expand in the next few years as more isolates are been sequenced. This information is important in order to understand the virus at the molecular level, determine possible virulence factors, and help develop future diagnostic and preventive tools.

Epidemiology

Unfortunately, little is known about the epidemiology of ECoV, as most studies have focused on individual outbreak reports. Until the early 2010s, ECoV was considered one of many enteric pathogens associated with foal diarrhea. ECoV was first recognized as an enteric pathogen of adult horses by Oue and collaborators.[26] Since then, ECoV has been recognized as an emerging virus from adult horses with fever and enteric signs in Japan, the USA, and Europe.[26-31,33-37] ECoV has also been reported in healthy adult horses from the USA, Saudi Arabia and Oman.[38,39]

Available demographic information has been retrieved from diagnostic laboratories showing that the overall number of ECoV qPCR-positive fecal samples has gradually increased since the introduction of the testing in 2012 (Real-time PCR Research and Diagnostics Core Facility, University of California, Davis, USA). The increasing frequency of qPCR-positive feces likely relates to greater awareness and testing availability. It is interesting to notice that submissions from ECoV qPCR-positive cases originate from all the lower 48 states of the USA. There is also an apparent seasonality to ECoV qPCR-positive cases with a higher detection rate during the colder months of the year (Figure 1),[27,40] which is similar to the seasonal disease pattern seen with the closely-related bovine coronavirus (BCoV).[41]

Outbreaks and individual cases of ECoV in adult horses have predominantly been reported in racing, pleasure and show horses and less frequently in breeding stocks. While age-susceptibility has been recognized for many animal and human coronaviruses, one hypothesis for the apparent low frequency of clinical ECoV cases in breeding equids is that ECoV is more likely to circulate between susceptible young and adult equids at breeding farms, leading to continuous silent exposure and protection against clinical disease.

However, observational exceptions are always present with biological processes and ECoV is no exception. There is one case report on an ECoV outbreak at a large American miniature horse breeding farm with 17% of breeding animals showing clinical disease.[36]

Prevalence factors associated with seropositivity to ECoV have been studied on 5,247 healthy adult horses originating from 18 states in the United States (Table 2).[42] A total of 504/5247 horses (9.6%) horses tested seropositive to ECoV using an S1-based enzyme-linked immunosorbent assay (ELISA). Geographic origin (Midwestern regions), breed (draft breed) and use (ranch/farm use, breeding use) displayed the highest odds ratio of seroprevalence. While breed predisposition to ECoV infection has not been determined, it is interesting to note that the initial and subsequent outbreaks from Japan all occurred in racing draft horses.[26,28] Another study evaluated the risk of exposure to ECoV in 333 apparently healthy horses from 29 farms throughout Israel using an S1-based enzyme-linked immunosorbent assay (Table 2).[43] A total of 41 out of 333 horses (12.3%) were seropositive. The ECoV seropositive horses originated from 17/29 farms (58.6%) and the seroprevalence per farm ranged from 0 to 37.5%. The only factor found to be significantly associated with ECoV exposure in the multivariable model was the geographical area, with a higher seroprevalence in horses residing in central Israel than in horses from the north or south. Longitudinal epidemiological studies are greatly needed in order to better understand and define risk factors associated with ECoV infection in adult equids.

Clinical Disease

In field outbreaks, the morbidity rates for ECoV infections in adult horses have been reported to range from 10 to 83%.[26-28,31,32] Fatalities are rare, but have been associated

with disruption of the gastrointestinal mucosal barrier leading to septicemia, endotoxemia and hyperammonemia-associated encephalopathy.[33,44]

The predominant clinical signs associated with ECoV in adult horses are non-specific and include anorexia, lethargy and fever (≥ 38.6 °C; Figure 2; Table 3).[26-28,31,33,34,36,37,40,45,46] While ECoV is an enteric virus, changes in fecal character (diarrhea to soft formed feces) and/or colic are not consistently observed, findings that often challenge equine practitioners into including ECoV as a differential diagnosis. Horses with ECoV infection can sometimes develop acute neurologic signs consistent with encephalopathy and are characterized by severe lethargy, head pressing, ataxia, proprioceptive deficits, recumbency, nystagmus and seizures.[27,33] It is speculated that the acute encephalopathic signs are caused by hyperammonemia, secondary to disruption of the gastrointestinal barrier.[44]

One must keep in mind that the majority of ECoV infections presenting to equine veterinarians may be mild and often self-limiting, requiring minimal to no medical care. However, horses with marked to severe systemic signs are often referred to equine veterinary hospitals and the severity of clinical disease may easily mimic other gastrointestinal diseases. In a retrospective case series of 33 adult horses testing qPCR-positive for ECoV in feces, the presenting complaints were fever (83%), anorexia (47%) and colic (43%).[40] When the hospitalized horses with qPCR-positive ECoV feces were compared to a cohort of horses with fever and/or loose manure that tested qPCR-negative for ECoV infection, presenting complaints were similar.[40] Twenty-seven ECoV qPCR-positive horses were hospitalized for a median of 5 days with 26/27 (96%) surviving to discharge.[40] Another study compared clinical features of ECoV infection with enteric

salmonellosis and found that the clinical signs of fever and colic were similar between the two groups.[45] Out of 8 horses classified as ECoV-positive, all survived; however, one horse developed clinical signs consistent with laminitis.[45] While most clinical ECoV infection are associated with outbreaks, some present as individual cases. A recent retrospective case series, including 5 horses with ECoV infection that were not associated with an outbreak, reported that anorexia and fever were observed in all horses and that 4 of 5 horses had moderate to severe diarrhea.[46] Patients were hospitalized for a median of five days and all survived to discharge. Collectively, the various case series using hospitalized horses have shown that clinical signs were not specific for ECoV and often similar to other enteric infectious diseases. However, the outcome of ECoV is favorable with the majority of the hospitalized horses surviving to discharge.

Experimental studies have shown that young and adult horses can successfully become infected with ECoV via the feco-oral route.[47-49] Because of the difficulty of growing ECoV *in vitro*, all experimental challenges used feces from naturally infected animals. Collectively, these studies have shown that the majority of infected horses develop mild and self-limiting clinical signs consistent with anorexia, fever and changes in fecal character (Table 4). Blood work showed leukopenia and/or lymphopenia in approximately half of the infected horses, which is consistent with hematological changes observed in naturally occurring cases of ECoV infection. ECoV was detected in the feces of all experimentally infected horse via qPCR, and nasal secretions and whole blood tested occasionally qPCR-positive for ECoV. While a qPCR-positive blood sample is consistent with viremia, it could not be determined if qPCR-positive nasal secretions were due to nasal replication and shedding of the virus, from environmental contamination from the

feces, or from both sources. While experimental infections with ECoV have only been attempted in a small number of horses, it appears that clinical disease, although mild, can be consistently reproduced, making the model suitable to study the pathogenesis and the immunity of ECoV.

Diagnosis

Diagnosing ECoV infection can be challenging, especially in horses lacking specific enteric signs. The diagnosis can be supported by the presence of neutropenia and/or lymphopenia and the detection of ECoV in feces.

Hematological findings of ECoV infections are generally consistent with a viral hemogram characterized by leukopenia due to neutropenia and/or lymphopenia. In a review of 35 cell blood counts from horses with natural ECoV infection supported via qPCR detection of coronavirus in feces, 74% of diseased horsed showed leukopenia, 66% neutrophilia and 72% lymphopenia (Figure 3). It is, however, important to keep in mind that approximately 10% of horses with ECoV infection display an unremarkable hemogram. Hematological abnormalities are expected to resolve within 5-7 days as long as no complications associated with the disruption of the gastrointestinal barrier occur. Less consistent hematological abnormalities can include the presence of band neutrophils, monocytosis and leukocytosis due to neutrophilia and monocytosis during the recovery period. When 8 clinical cases of ECoV infection were compared to 12 horses with enteric salmonellosis, neutrophil count was decreased in both groups but was not significantly different.[45] Further, in a case series of 33 horses with ECoV infection seen at a veterinary hospital, ECoV qPCR-positive horses had lower white blood cells (range 680 to 16200/µl,

median 3000/µl), neutrophil counts (150 to 14400/µl, median 1250/µl) and lymphocyte counts (420 to 3470/µl, median 860/µl) when compared to ECoV qPCR-negative horses.[40]

Serum biochemistry profiles may be unremarkable; however, abnormalities in ECoV infected horses have been reported and include electrolyte derangements, hyperbilirubinemia, hyperglycemia, hyperlipidemia, hypoproteinemia, increased muscle enzymes and azotemia.[40] ECoV infection with concurrent signs of encephalopathy has been linked to hyperammonemia. A recent ECoV case series reported on one horse with severe hyperammonemia (677 µmol/L; reference interval ≤ 60 µmol/L) with encephalopathic signs that subsequently died [REF?]. Hyperammonemia associated with ECoV infection is likely due to increased ammonia production within or absorption from the gastrointestinal tract due to gastrointestinal barrier breakdown. An increase in enteric ammonia production could also be the result of bacterial microbiome changes associated with ECoV infection.

It is only in recent years that the diagnostics for ECoV have markedly improved with the use of qPCR. The limitation of historical detection modalities, such as negative stain electron microscopy (EM) or antigen-capture ELISA, is that they are not sensitive enough when viral particles are not present in sufficient numbers. The biological sample of choice to support an ECoV diagnosis are feces or rectal swabs. Previous work has shown that molecular assays are rapid, cost-effective, sensitive and specific for ECoV.[27,36,50] Unfortunately, no study has compared the various molecular techniques (qPCR versus RT loop-mediated isothermal amplification) and their superior sensitivity to negative stain EM or antigen-capture ELISA. While the detection of ECoV in the feces of diseased horses is highly suggestive of infection, one must keep in mind that 4 to 83% of horses can remain

subclinically infected during an ECoV outbreak.[27,33,36,37] Viral kinetics of ECoV in feces from experimentally infected horses have shown that horses begin to shed detectable ECoV RNA in their feces at 3 or 4 days post-infection and continue shedding virus until 12 or 14 days post-infection.[47-49] Peak ECoV shedding is consistently seen on day 3 to 4 following the development of clinical disease (Figure 4). Average length of ECoV RNA detection following onset of natural infection ranges from 3-9 days, however, naturally infected horses have been shown to sporadically shed ECoV RNA in feces up to 98 days.[27,33,36,37] While additional biological samples such as whole blood (viremia) and nasal secretions (shedding) have tested qPCR-positive for ECoV in experimentally[47-49] and naturally[29,51,52] occurring infections, these sample types do not consistently test positive and should not be used to support a diagnosis of ECoV infection. The molecular detection of ECoV can be challenging, especially during peracute disease when diseased horses experience gastrointestinal stasis due to colic and/or there are not enough viral particles in the feces to be detected. Recommendations are to repeat testing of fecal matter in a suspected index case at a later time point or collect multiple samples for pooled testing. Further, it has been the author's experience that ECoV infected horses presenting with systemic clinical signs such as fever, lethargy and anorexia are more likely to be tested for respiratory pathogens via respiratory secretions than enteric pathogens through feces. In order to speed up diagnostic turn-around-time, one should consider submitting both, nasal secretions and feces, in adult horses with acute onset of systemic signs in order to test for selected respiratory and enteric pathogens or stage the testing to the more likely etiology. This process will speed up the analysis as additional samples (i.e., feces) have already been shipped to the laboratory and are available for ECoV testing.

Serology has been established and validated for ECoV and is available in research laboratories mostly to document seroconversion in experimentally infected horses or study the epidemiology of ECoV in various horse populations.[26,28,42,43,47,48,53,54] Serology can be used to retrospectively establish recent infection using acute and convalescent serum samples and documenting either seroconversion or increase in serum titers.

Necropsy cases of suspected enteritis should have feces or gastrointestinal content tested by qPCR for ECoV and other gastrointestinal infectious agents. Further, formalin-fixed intestinal tissue samples can also be tested by immunochemistry and direct fluorescent antibody testing using BCoV reagents.[44]

Pathogenesis

Following an incubation period of 48-72 hours, most horses infected with ECoV develop a self-limiting enteritis, which generally resolves with minimal supportive care. In the vast majority of clinical adult horses, ECoV is the only pathogen detected in the feces of affected horses, suggesting a unique pathogenicity.[27,28,40] However, little is known about the pathogenesis of ECoV, other than its tropism to enterocytes.[55,56] A recent case series reported on the pathology of ECoV infection in three naturally infected equids with sudden death.[44] Gross and histological findings were consistent with severe diffuse necrotizing enteritis, characterized by marked villus attenuation, epithelial cell necrosis in the tips of the villi, neutrophilic and fibrin extravasation into the small intestinal lumen, as well as crypt necrosis, microthrombosis, and hemorrhage. In these three necropsied cases, ECoV was detected by qPCR in intestinal tissue, gastrointestinal content, and/or feces. Further, coronavirus antigen was detected by immunohistochemistry and/or direct fluorescent antibody testing in the small intestine of all cases. In comparison to BCoV, there is no

evidence that ECoV has respiratory tropism based on the lack of respiratory signs in affected equids and histological changes in horses undergoing necropsy.[57] The lack of respiratory tropism is supported by a recent experimental study, which showed that in only 1 out of 4 horses, ECoV was detected in the lungs by qPCR but not by in-situ hybridization.[58] Apparently, lung cells themselves were not susceptible to ECoV and qPCR-positive lung tissue was caused by viremia.[58]

One rare complication of clinical ECoV has been the development of often-fatal encephalopathy, suspected to occur secondary to hyperammonemia.[33] In the case series reporting on the pathology of ECoV infection in three naturally infected equids, one of them displayed hyperammonemic encephalopathy with Alzheimer type II astrocytosis throughout the cerebral cortex, suggesting a strong association between ECoV necrotizing enteritis, hyperammonemia and the development of encephalopathy.[44]

Therapeutic Strategies

The majority of horses with clinical ECoV infection recover in a few days with little supportive to no treatment at all. Horses showing persistent elevated rectal temperature affecting their appetite and attitude have been treated with antipyretic (dipyrone at 30 mg/kg BWT q12-24 h IV) or anti-inflammatory drugs (flunixin meglumine (0.5-1.1 mg/kg BWT q12-24 h IV or PO); phenylbutazone (2-4 mg/kg BWT q12-24 h IV or PO); firocoxib (0.1 mg/kg BWT q24 h PO or 0.09 mg/kg BWT q24 h IV)) for 24 to 48 hours, as long as they stay hydrated. Horses with colic, persistent anorexia and/or diarrhea have been treated more intensively with fluid and electrolytes per nasogastric intubation or intravenous administration of polyionic fluids until clinical signs have resolved. Additionally, antimicrobials and gastrointestinal protectants should be considered in horses

developing signs of endotoxemia and/or septicemia secondary to disruption of the gastrointestinal barrier. Horses with suspected or documented hyperammonemia should be treated with oral lactulose (0.1-0.2 ml/kg BWT q6-q12 h PO), neomycin sulfate (4-8 mg/kg BWT q8 h PO) or fecal transfaunation and crystalloid fluids.

Immunity and Immunoprophylaxis

Little is known about the immune responses and immune protection against ECoV. For the closely-related BCoV, serum levels of neutralizing and HI antibody from naturally infected calves and cattle arriving at feedlots have been shown to correlate with protection against both, enteric and respiratory disease.[59-62] Further, cattle that recovered from winter dysentery after experimental infection with BCoV maintained a very long-lasting BCoV-specific serum (IgA and IgG) and local (IgA) antibody response.[63] It will need to be determined if certain levels of antibodies and what type of specific immune response correlates with protection against ECoV.

Immunization strategies have been best described in cattle for the prevention of winter dysentery infection using commercially available BCoV vaccines. Due to the close genetic homology of ECoV with BCoV, serological responses to BCoV vaccines have recently been investigated in horses. One study used a killed-adjuvanted BCoV vaccine in six healthy yearling horses and reported a measurable serological response in all horses following the administration of two vaccines given 28 days apart.[64] A second study investigated the safety, humoral response and viral shedding in horses inoculated either orally, intranasally or intrarectally with a commercially available modified-live BCoV vaccine.[65] The results of that study showed that the modified-live BCoV was safe to administer to horses via various routes, caused minimal virus shedding and resulted in

detectable antibodies to BCoV in 27% of the vaccinates. Collectively, these two BCoV vaccines, while showing measurable antibody responses to BCoV, cannot be recommended at the present time due to the lack of efficacy data.

Control Strategies

The cornerstone of ECoV prevention resides in strict biosecurity measures aimed at reducing the risk of introducing and disseminating ECoV on equine premises. It is important to be vigilant when working-up horses presenting with fever, anorexia and lethargy, with or without concurrent enteric signs. Such horses should be isolated until ECoV, as well as other potential infectious pathogens, have been ruled in or out. ECoV qPCR-positive horses should be isolated and stable- or herd-mates closely monitored until the outcome of past-exposure has been determined. Outbreaks of ECoV are generally short lasting, especially when strict biosecurity measures have been followed, and quarantine can routinely be lifted 2-3 weeks following the resolution of clinical signs in the last affected horse.

Coronaviruses are susceptible to heat, detergents and disinfectants like sodium hypochlorite, povidone iodine, 70% ethanol, glutaraldehyde, quaternary ammonium compounds, phenolic compounds, formaldehyde, peroxymonosulfate, and accelerated hydrogen peroxide.[66-69] Moreover, coronaviruses have been reported to survive well at low temperatures and high relative humidity. Their survival on surfaces is also long, up to 120 h and even longer in organic medium such as feces, urine and wastewater.[70-73]

Summary

As incidental hosts, horses are unlikely to contribute to the spread of SARS-CoV-2. Rare infections of SARS-CoV-2 have been reported in equids, thought to occur secondary to the spillover of SARS-CoV-2 from symptomatic or asymptomatic COVID-19 individuals. ECoV has emerged as an enteric pathogen of adult horses in recent years and has been reported in Japan, Europe and the USA. There are increasing reports of the disease, arising from increased awareness in the field and the availability of diagnostic tests for detecting ECoV in feces of affected horses. Clinical presentation of ECoV infection is often limited to systemic signs such as fever, lethargy and anorexia, while enteric signs are present in less than 20% of infected cases. While blood work may suggest a viral infection (lymphopenia and neutropenia), laboratory diagnosis is supported by the detection of ECoV in feces using qPCR. ECoV infection is often self-limiting, requiring little to no supportive treatment. While no vaccine is available at the present time, prevention of ECoV infection is best achieved though routine management practices aimed at reducing the likelihood of introducing and disseminating ECoV at any horse-based premise as well as the timely isolation of horses with suspected clinical ECoV infection.

Disclosure

The author has nothing to disclose.

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Legend to Figures

Figure 1. Monthly distribution of ECoV qPCR-positive fecal samples amongst all fecal samples submitted to a private diagnostic laboratory in the USA. The data encompasses the time frame from January of 2012 to May of 2022.

Figure 2. Compilation of clinical signs observed from 101 adult horses with clinical ECoV infection (Pusterla, personal communication). Fever was defined as a rectal temperature ≥ 38.6 °C. Neurological signs were consistent with encephalopathy and included aimless wandering, circling, head pressing, recumbence and seizure.

Figure 3. White blood cell, neutrophil and lymphocyte count of 35 adult horses with suspected ECoV infection. The results are expressed as individual values. Median values are represented by horizontal bars. Red brackets represent normal reference ranges for each cellular fraction displayed (WBC 5000 to 11600/µl; neutrophil count 2600 to 6800/µl; lymphocyte count 1600 to 5800 µl).

Figure 4. Diagram showing temporal clinical signs and fecal shedding of ECoV in an adult horse presented to a referring hospital because of anorexia, lethargy and fever.

Table 1: Studies documenting the susceptibility of equids to SARS-CoV-2.

Study type	Equid (number)	Outcome	References
Experimental infection	Horse (1)	No molecular detection of SARS-CoV-2 in nasal secretions and feces and no virus isolation from respiratory tissues	15
Direct contact to breeders/keepers with COVID-19	Horse (34)	No detection of SARS-CoV-2 in respiratory secretions and/or feces	17
Horses with acute onset of fever and respiratory signs	Horse (667)	No detection of SARS-CoV-2 in nasal secretions	18
Serology healthy horses from China	Horse (18)	No specific antibodies against SARS-CoV-2 detected	20
Serology racing horses in contact with COVID-19 track workers	Horse (587)	Antibodies against SARS-CoV-2 detected in 35/587 horses (5.9%)	18
Healthy horses in contact with COVID-19 horse owner	Horse (2)	No detection of SARS-CoV-2 in nasal secretions, blood and feces, one horse seroconverted against SARS-CoV-2	19

Table 2. Seroprevalence and risk factors for ECoV documented in populations of healthy adult horses in the USA and Israel.

Population	Seroprevalence	Country	Risk factors	References
Healthy horses (n=5247)	9.6%	USA	Midwestern regions (P = 0.008) Draft horse breed (P = 0.003) Ranch/farm use (P = 0.034) Breeding use (P = 0.016)	42
Healthy horses (n=333)	12.3%	Israel	Northern regions (P < 0.001)	43

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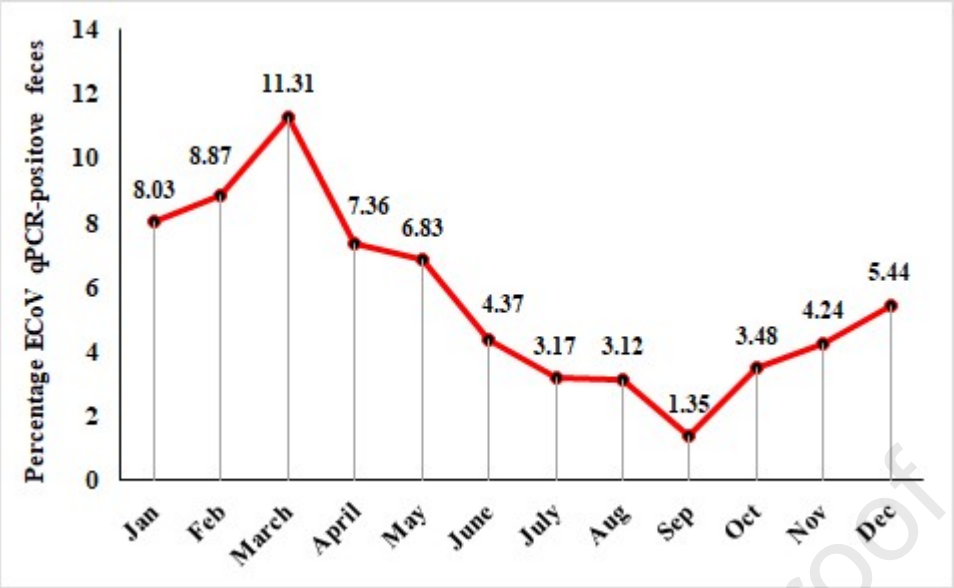
Table 3. Studies documenting clinical signs associated with natural ECoV infection in adult horses.

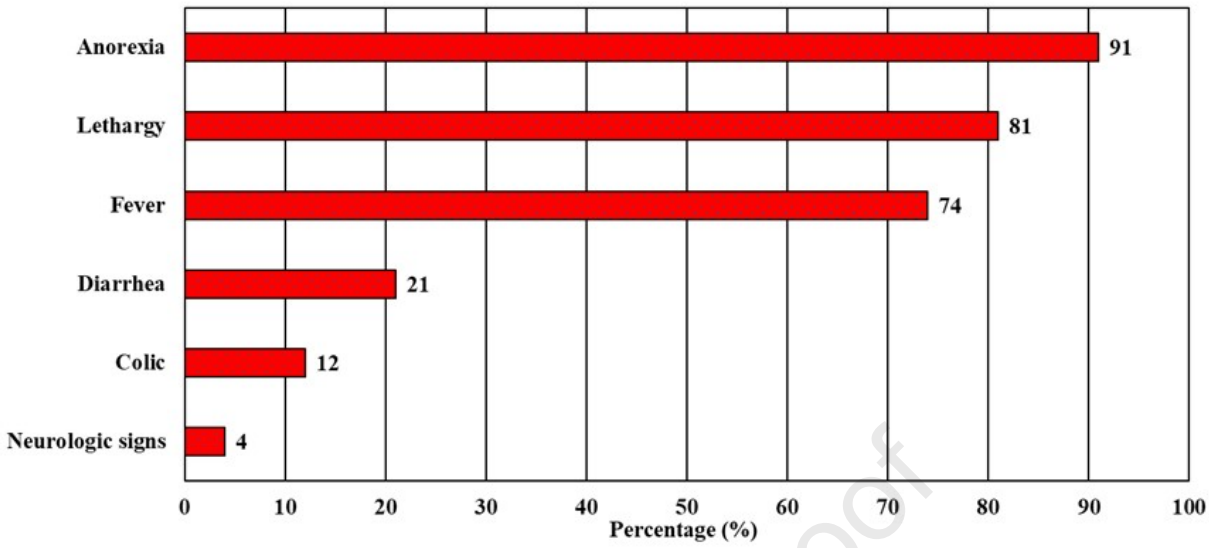
Population (morbidity)	**Age in years (median)**	**Country**	**Clinical signs (percentage of reported signs)**	**References**
Race horses (132/600 diseased horses)	2-4	Japan	Fever, diarrhea	26
Riding horses (59/165 diseased horses)	1-29 (15)	USA	Anorexia (88.1%), lethargy (77.9%), fever (72.8%), diarrhea (20.3%), colic (6.8%), neurological signs (3.4%)	27
Race horses (204/650 diseased horses)	2-11 (3)	Japan	Fever (96.1%), anorexia, diarrhea (10.8%), colic (3.9%)	28
Pleasure horses (7/26 diseased horses)	8-25 (18)	Switzerland	Fever (85.7%), anorexia (85.7%), cecal impaction (14.3%), diarrhea (14.3%)	31
Miniature horses and donkey (15/27 diseased equids)	0.5-19 (6)	USA	Fever, lethargy, anorexia, colic, neurological signs	33
Adult Thoroughbred (1 diseased horse)	19	United Kingdom	Fever, lethargy, anorexia, colic	34
Thoroughbred (3 diseased horses)	Yearling		Lethargy, weight loss (concurrent larval cyathostomiasis)	

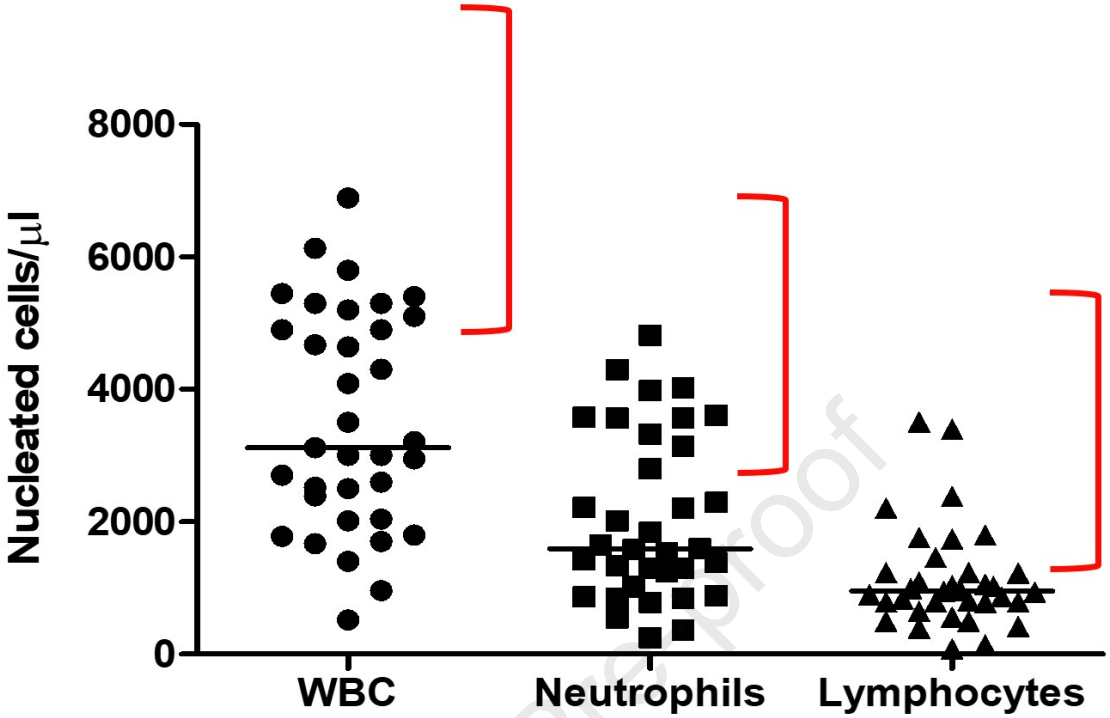
American miniature horses (5/30 diseased horses)	0.5-12 (5)	USA	Anorexia (100%), fever (100%), lethargy, colic (40%), diarrhea (20%)	36
Adult riding horses (15/41 diseased horses)	1-19 (10.8)	Japan	Anorexia (27%), fever (73%), lethargy (40%), diarrhea (20%)	37
Hospitalized adult horses (33 diseased horses)	2-37 (11)	USA	Fever (83%), anorexia (47%), colic (43%), lethargy (27%), diarrhea (3%), foot soreness (3)	40
Hospitalized adult horses (8 diseased horses)	3-16 (6.5)	USA	Fever (50%), lethargy (25%), anorexia (12.5%), colic (12.5%), diarrhea (25%)	45
Hospitalized adult horses (5 diseased horses)	8-13 (9)	USA	Fever (100%), anorexia (100%), lethargy (60%), colic (40%), diarrhea (20%)	46

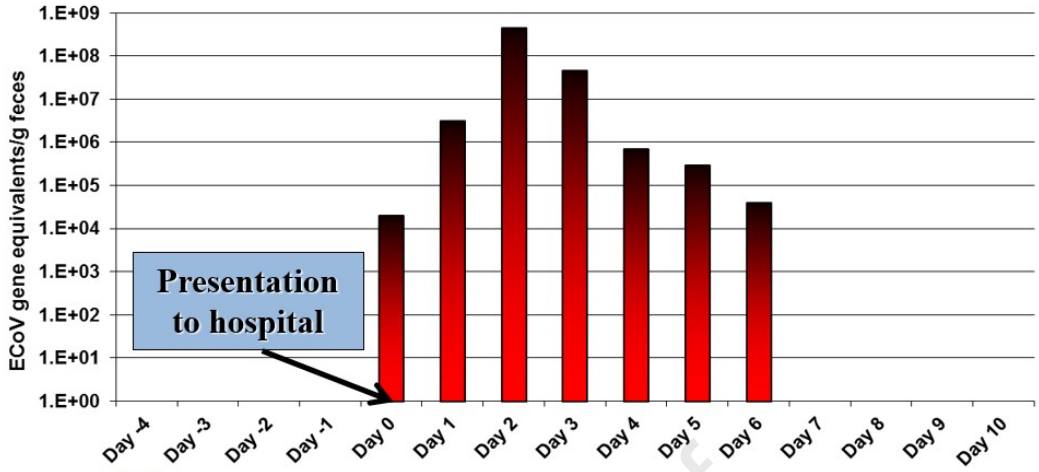
Table 4. Studies documenting clinical, hematological and laboratory findings associated with experimental ECoV infection in young and adult horses. The number of horses for each of the parameters is listed in parenthesis.

Population (number of horses)	**Clinical signs**	**Blood work**	**Molecular testing**	**Serology**	**References**
9-10-month-old draft horses (3)	Fever (2), anorexia (2), pasty feces (2)	Leukopenia/lymphopenia (1), elevated SAA (2)	ECoV RNA in feces (3), nasal secretions (3) and blood (2)	Seroconversion (3)	47
Adult horses (8)	Gastrointestinal hypermotility (7), loose manure (7), fever (1)	Lymphopenia (4)	ECoV RNA in feces (8), nasal secretions (4) and blood (1)	Seroconversion (4)	48
Yearling Thoroughbred horses (4)	Fever (2)	Not applicable	ECoV RNA in feces (4), nasal secretions (3) and blood (2)	Not applicable	49









Possible Exposure

Lethargy/anorexia

Fever (> 101.5°F)

Soft formed feces

Hospitalization

CBC: leukopenia (950/µl), neutropenia (650/µl), lymphopenia (240/µl)