Corona Virus in Horses: Any Reason to Panic ?

Dr. Nicola Pusterla, Diplomate ACVIM and AVDC-Equine

Coronaviruses are single-stranded, positive sense, non-segmented, enveloped RNA viruses belonging to the *Coronaviridae* family, with four genera defined based on serological cross-reactivity and genetic homology: *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus* and *Gammacoronavirus* [1]. ECoV is classified within the *Betacoronavirus 1* genus, along with human coronaviruses OC43, 4408 and HKU1, bovine coronavirus (BCoV), porcine hemagglutinating encephalomyelitis virus, canine respiratory coronavirus, mouse hepatitis virus, bubaline coronavirus and sialodacryoadenitis rat coronavirus [2]. ECoV is genetically distinct from the human SARS-CoV-2 and there is no evidence to indicate that horses could contract SARS-CoV-2 or that horses may be involved in the spread of SARS-CoV-2 to other animals or humans.

Almost 11 years ago, a research group investigated an unusual outbreak of fever and enteric signs in 2- to 4-year-old racing draft horses in Tokachi, Hokkaido, Japan [3]. It is of interest to notice that enteric signs were only reported in 10% of the horses, and a total of 132/600 horses (22%) became diseased. The same racing venue experienced one additional outbreak with similar signs three years following the first outbreak [4]. Additional outbreaks have since been observed and reported in the USA and Europe [5-8]. Collectively, these outbreaks have been able to refine the clinical presentation of ECoV, one that is still perplexing considering the inconsistent development of enteric signs. The lack of enteric signs such as colic and/or changes in fecal character may specifically relate to the intestinal section affected by the virus. ECoV has

been shown to cause enteritis in both foals and adult horses [9,10]. While enteritis is consistently associated with diarrhea in foals, this condition may not affect the fecal character of infected adult horses. Horses infected with ECoV generally recover with minimal to no medical treatment within 2-4 days post-onset of clinical signs. While ECoV infection is often self-limiting, adult horses may occasionally require intensive care to resolve leukopenia, systemic inflammation and metabolic disturbances [11, 12]. Morbidity rates have been reported to range between 17-57% [3-5, 13] and there are still yet undetermined host, viral, and environmental factors that impact susceptibility and outcome of ECoV infection. Of interest is the observation that clinical expression of ECoV infection is age-dependent with foals rarely developing clinical disease. Given the lack of documented outbreaks of ECoV at large breeding farms, it is possible that virus circulating between foals and breeding stock confers protection against clinical disease. This hypothesis is supported by a recent study documenting a higher seroprevalence to ECoV in healthy breeding animals compared to non-breeding horses [14]. Age-related patterns of disease have been reported with other coronaviruses such as feline coronavirus (FCoV), with most feline infectious peritonitis (FIP) cases occurring in young animals while older cats often experience a protracted disease. It is often the naïve and adaptive immune responses that modulate disease expression and in the case of FCoV, a weakened cell-mediated immune response is at the origin of fatal infections [15]. While immune-mediated mechanisms may also be responsible for the age-related disease expression of coronavirus infection in horses, future studies will need to characterize how the innate immune systems battles ECoV.

It is the lack of gastrointestinal signs that often misleads the equine veterinarian into ruling out an enteric pathogen. Leukopenia due to neutropenia and/or lymphopenia is a consistent

hematological abnormality and, although not specific for ECoV, should direct the diagnostic work-up toward a viral disease. The laboratory support of ECoV infection is based on the detection of the virus in feces. Historical detection modalities such as electron microscopy and antigen capture ELISAs have been supplanted by quantitative real-time PCR (RT-qPCR). RT-qPCR has the advantage of being highly sensitive and specific, has a quick turn-around-time and is cost-effective. Further, the quantitative capability of RT-qPCR allows the study of viral kinetics and the ability to determine the horse's contagious nature and prognosis. Experimental studies have shown that ECoV RNA can be detected as early as 72-96 hours post-inoculation and continues to be detected until 10-14 days post infection [16, 17]. In naturally infected horses, ECoV can be detected by RT-qPCR for 3-9 days with a detection time occasionally extending up to 25 days from onset of clinical disease [5, 13, 18]. Although the number of experimentally infected horses with ECoV reported in the literature is small, it appears that longer duration and higher peaks of viral shedding are observed in clinically versus non-clinically infected horses. This observation is, however, in sharp contrast to viral loads of naturally infected horses with and without clinical signs, although, in the latter populations, the results originated from a single time point and did not include follow-up results [5]. One can only assume that during an outbreak, horses with and without clinical signs may have similar ECoV viral kinetic patterns and both might contribute to environmental contamination and viral transmission. While several factors such as viral strain, age of patient and co-morbidity influence the outcome of coronavirus infection, a recent study was able to associate ECoV viral loads measured by RT-qPCR with mortality [18]. Similarly, in SARS-CoV infections, patient survival during acute disease is correlated with viral load [19, 20]. One factor affecting viral load is the ability of individuals to

express age-dependent genes involved in inflammation and innate immunity [20]. The latter mechanism is specific for various viruses and highlights the virus/host interaction in disease severity and outcome.

Complications associated with ECoV are rare and have been associated with disruption of the gastrointestinal mucosal barrier leading to endotoxemia, septicemia and hyperammonemia-associated encephalopathy [5, 18]. Due to the rapid autolysis of the gastrointestinal tract, it is relevant to have a necropsy performed rapidly or have representative samples collected and frozen for ECoV detection and placed in formaldehyde for histological evaluation. Histological changes have only been reported in a very small number of horses and showed morphological changes similar to BCoV infection [10]. The histological hallmarks of ECoV infection were diffuse necrotizing enteritis, marked villus attenuation, epithelial cell necrosis of the tips of the villi, neutrophilic and fibrin extravasation into the small intestinal lumen, as well as crypt necrosis, microthrombosis and hemorrhage [10]. Post-mortem diagnosis of ECoV can be achieved by RT-qPCR on feces or small intestinal contents, and ECoV can be detected in intestinal tissue by electron microscopy, immunochemistry and direct fluorescent antibody testing using BCoV reagents [10].

Specific preventive measures are scarce, and there are yet no licensed vaccines against ECoV. Due to the close genetic homology of ECoV with BCoV, serological responses to BCoV vaccines have recently been investigated. 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 [21]. 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 [22]. 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 this time due to the lack of efficacy data. 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 herdmates 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. While common disinfectants inactivate ECoV, it is unknown as to how long ECoV remains infectious in the environment. Severe acute respiratory syndrome (SARS)-CoV has been shown to persist up to 2 days in wastewater and dechlorinated tap water, 3 days in feces and 17 days in urine at room temperature [23]. The survival of the virus is even longer at lower temperatures.

In conclusion, equine veterinarians should take advantage of the breadth of knowledge and experience gained over the past decade in the field of ECoV of adult horses. While various aspects of this virus are still in need of investigation, equine veterinarians are well-armed with an arsenal of clinical observations, diagnostic tools and treatment modalities that should guarantee a

successful outcome of horses infected with ECoV.

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Contact Information

Department of Veterinary Medicine and Epidemiology

School of Veterinary Medicine, University of California

One Shields Avenue, Davis, California, USA

E-mail: npusterla@ucdavis.edu