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Seroprevalence and risk factors for infection with equine coronavirus in healthy horses in the USA

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ABSTRACT

Equine coronavirus (ECoV) is considered an enteric pathogen of foals and has only recently been associated with infections in adult horses. Seroprevalence data is needed to better understand the epidemiology of ECoV in adult horses, evaluate diagnostic modalities and develop preventive measures. The objective of this study was to investigate the seroprevalence and selective risk factors for ECoV in 5247 healthy adult horses in the USA, using a recently established and validated IgG enzyme-linked immunosorbent assay. Prevalence factors analysed in this study included geographic region, age, breed, sex and use.

A total of 504/5247 horses (9.6%) horses tested seropositive. Geographic region (Mid-West; $P = 0.008$), breed (Draft horses; $P = 0.003$) and specific uses of horses (ranch/farm, $P = 0.034$; breeding use, $P = 0.016$) were all statistically significant risk factors for seropositivity.

Introduction

Coronaviruses are part of the coronaviridae family, which are positive-strand RNA viruses. The coronaviridae subfamily is grouped into four genera, based on genetic differences and serological cross-reactivity (Woo et al., 2009, 2012). Equine coronavirus (ECoV) is a β-coronavirus, as are human coronavirus OC43 and HKU1, murine hepatitis virus, bovine coronavirus (BCoV), porcine haemagglutinating encephalomyelitis virus, canine respiratory coronavirus and rat coronavirus (Zhang et al., 2007). ECoV is considered an enteric pathogen of foals (Guy et al., 2000) and has only recently been associated with infections in adult horses in Japan, the USA and Europe (Oue et al., 2011, 2013; Pusterla et al., 2013; Miszczak et al., 2014). Reported morbidity rates during outbreaks range from 20–83% (Pusterla et al., 2013; Fielding et al., 2015; Giannitti et al., 2015).

Despite the sporadic occurrence of ECoV outbreaks in adult horses, the overall seroprevalence of this virus in horse populations remains largely unknown. Seroprevalence data is needed to better understand the epidemiology of ECoV, evaluate diagnostic modalities and develop preventive measures. Therefore, the objective of this study was to investigate the seroprevalence and selective risk factors to ECoV in 5247 healthy adult horses originating from 18 US states using a recently established and validated ELISA (Kooijman et al., 2016).

Materials and methods

Study population

The study population consisted of 5247 healthy horses from 18 states (CA, CO, FL, KY, MD, MN, MS, MO, NY, NC, OH, PA, TN, TX, VA, WA, WI, WY) representing the four regions of the USA (North-East, South, Mid-West, West). States sampled from the four regions were as follows: North-East (NY, MD, PA); South (FL, MS, KY, TN, NC, VA, TX); Mid-West (MO, OH, WI, MN); West (WA, CA, CO, WY). Banked sera collected by Zoetis USA for the purposes of another study were used. Blood samples were collected by convenience sampling from healthy horses by equine veterinarians from participating veterinary clinics across the USA during the autumn of 2013. Clinics enrolled voluntarily and each clinic sampled approximately 110 horses; no more than 10 horses sampled from each clinic resided on the same farm. Jugular venipuncture was used to collect 10 mL of whole blood from each horse. Additionally, age, breed, primary use, and sex data were collected for each horse by questionnaire. Blood samples were centrifuged, serum was separated, shipped on ice, processed and stored at –20 °C.

Serological analysis

All sera were tested for IgG to ECoV using an ELISA based on a recombinant protein containing two immunodominant areas of the spike protein of ECoV, including the area with the highest predicted antigenic sequence (Kooijman et al., 2016). Briefly, microtiter plates were coated with 0.0156 µg recombinant protein per well and sera were tested at a dilution of 1 µL serum in 100 µL of buffer. Each plate contained known negative and positive controls. Negative controls consisted of sera collected from healthy adult horses from the university herd with no history of systemic disease and qPCR-negative feces for ECoV. Positive controls consisted of sera collected from horses 30 days after they developed clinical signs compatible with ECoV infection and tested qPCR-positive for ECoV in the faeces. A secondary horseradish-peroxidase labelled IgG was used to bind with ECoV-specific antibodies from the serum samples. An enzyme substrate (Bethyl ELISA starter accessory kit II) was used to detect the secondary antibody. The optical density (OD) of each well was measured and cor-

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Table 1
Frequency of equine coronavirus (ECoV) seropositivity per state as defined by ELISA in 5247 equine sera collected from 18 different states.

State (*n*)	ECoV positive samples (%)
Maryland (100)	4 (4)
Colorado (321)	15 (5)
Virginia (262)	13 (5)
Pennsylvania (220)	13 (6)
Kentucky (328)	22 (7)
Washington (320)	21 (7)
Florida (327)	25 (8)
New York (330)	25 (8)
Tennessee (220)	18 (8)
Texas (330)	26 (8)
California (328)	30 (9)
Missouri (330)	34 (10)
North Carolina (330)	32 (10)
Ohio (320)	34 (11)
Wyoming (419)	53 (13)
Mississippi (110)	17 (15)
Wisconsin (322)	57 (18)
Minnesota (330)	65 (20)

rected by subtracting the average of the OD values of the negative control samples. Sera were considered positive if the corrected value was above the average plus three times the standard deviation of the OD of the negative control samples (Kooijman et al., 2016).

Statistical methods

Risk factors analysed in this study included geographic regions (North-East, South, Mid-West, West), age, breed (Quarter horse, Warmblood, Thoroughbred, Paint horse, Arabian, Draft horse, Pony/miniature, other), sex (female, gelding, male) and use (competition, ranch/farm, breeding, other). Age was analysed in 5-year increments as a categorical variable.

Univariate logistic regression (controlling for the random effect of farm of origin) of each prevalence factor was performed to determine the odds ratios associated with region, age, breed, sex and use. Further, a mixed effects logistic regression model was developed to include significant risk factors and the random effects parameter of horses originating from the same farm. All statistical evaluations analyses were performed in Stata 12 (Stata statistical software, Version 12) and statistical significance was set at $P < 0.05$.

Results

The total number of ECoV positive horses was 504/5247 (9.6%). The seroprevalence per state ranged from 4.0–19.7% (Table 1). Fig. 1 shows the number of horses sampled in each region and the rates of seropositive samples (%). Horses aged >20 years (13.0%), Draft horses (17.6%) and ranch/farm horses (12.0%) had the highest seroprevalence (Table 2). The median age of the study population was 11 years (range, 2–40 years).

Statistically significant risk factors in the mixed effects univariate analysis included region (Mid-West), age (>20 years old), breed (Draft horse, Thoroughbred) and use (ranch/farm). There was no significant difference in risk for ECoV seropositivity between male and female horses.

In the mixed effects multivariate model, horses from the Mid-West displayed the highest odds ratio of seropositivity in the region category when compared to the reference region (Table 3). Draft horse showed the highest odds ratio of seroprevalence in the breed category, while Thoroughbreds had the lowest odds ratio of seroprevalence. Ranch/farm and breeding horses displayed the highest odds ratio of seroprevalence in the use category. There was no significant difference between male and female horses and between the reference age group and the other age groups.

The total number of ECoV negative horses was 4743/5247 horses (90.4%). Table 2 shows the number (%) of seropositive and seronegative horses sampled for each of the risk factor categories.

In the univariate model, horses aged 1–5 years old had 91.7% seronegativity, Thoroughbreds had the highest % seronegativity (95.1%) and competition horses had the highest % seronegativity (92.4%).

In the mixed effects multivariate model, horses from the Mid-West were least likely to be seronegative, Thoroughbreds were most likely to be seronegative and Draft horses were least likely to be seronegative (Table 3). There was no significant difference in odds for seronegative status by age group, sex and use.

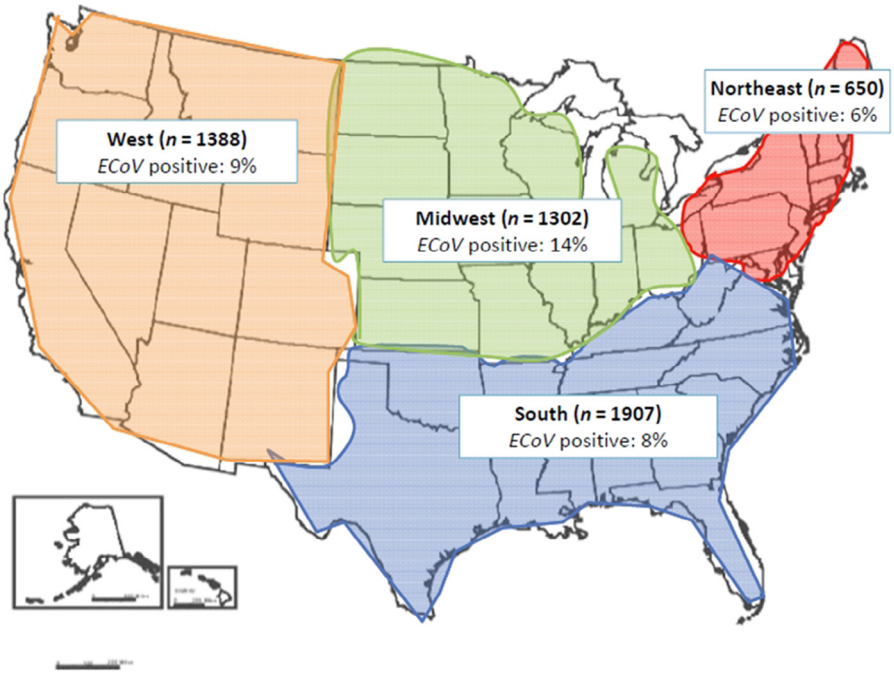

Fig. 1. Frequency of equine coronavirus (ECoV) seropositivity by ELISA per region in 5247 equine sera collected across the USA.