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Diagnostic approaches, aetiological agents and their associations with short-term survival and laminitis in horses with acute diarrhoea admitted to referral institutions

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Abstract

Background: An international description of the diagnostic approaches used in different institutions to diagnose acute equine diarrhoea and the pathogens detected is lacking.

Objectives: To describe the diagnostic approach, aetiological agents, outcome, and development of laminitis for diarrhoeic horses worldwide.

Study design: Multicentre retrospective case series.

Methods: Information from horses with acute diarrhoea presenting to participating institutions between 2016 and 2020, including diagnostic approaches, pathogens detected and their associations with outcomes, were compared between institutions or geographic regions.

Results: One thousand four hundred and thirty-eight horses from 26 participating institutions from 4 continents were included. Overall, aetiological testing was limited (44% for *Salmonella* spp., 42% for *Neorickettsia risticii* [only North America], 40% for *Clostridiodes difficile*, and 29% for ECoV); however, 13% (81/633) of horses tested positive for *Salmonella*, 13% (35/262) for *N. risticii*, 9% (37/422) for ECoV, and 5% (27/578) for *C. difficile*. *C. difficile* positive cases had greater odds of non-survival than horses negative for *C. difficile* (OR: 2.69, 95%CI: 1.23–5.91). In addition, horses that were positive for *N. risticii* had greater odds of developing laminitis than negative horses (OR: 2.76, 95%CI: 1.12–6.81; $p = 0.029$).

Main limitations: Due to the study's retrospective nature, there are missing data.

Conclusions: This study highlighted limited diagnostic investigations in cases of acute equine diarrhoea. Detection rates of pathogens are similar to previous reports. Non-survival and development of laminitis are related to certain detected pathogens.

KEYWORDS
antimicrobial associated diarrhoea, Clostridiosis, horse, Potomac horse fever, sand diarrhoea

The Multicenter Equine Diarrhea Study group (see names and affiliations in Supplementary Item 1).

1 | INTRODUCTION

Acute diarrhoea is one of the leading causes of hospitalisation and mortality in horses worldwide. Although establishing an aetiological diagnosis is essential for facilitating well-defined biosecurity, treatment and prognostic recommendations, reaching a diagnosis is often challenging.[1] Common causes of acute equine diarrhoea include *Salmonella* enterica spp., *Clostridoides difficile*, Equine coronavirus (ECoV), *Neorickettsia risticii* (Potomac horse fever), cyathostomes, sand enteropathy, antimicrobial-associated diarrhoea (AAD) and non-steroidal medication induced right dorsal colitis.[2] However, the cause of diarrhoea is identified in fewer than 50% of horses even after extensive diagnostic testing (e.g., necropsy, histopathology, bacterial cultures, PCR, parasitology toxicology).[1,3]

The prevalence of sand accumulation and of each pathogen associated with acute diarrhoea in horses appears to vary by institution, season, geographical location, and population studied.[4–9] There is currently little information available regarding the differences in pathogen detection beyond single centre studies[4,5,9–12] or multicentre studies from a single country.[7] A single study reported pathogen detection from diagnostic laboratories receiving specimens from a wider region without precise information on the health status of the horses.[13] A comprehensive description of the diagnostic approaches used in different institutions for the diagnosis of equine diarrhoea and the pathogens detected worldwide is lacking. Identifying the pathogens detected in each geographic area can provide critical information to design cost-effective guidelines or protocols for pathogen testing at different institutions.[1,7]

Disease severity, outcome, and occurrence of complications, such as laminitis, have been linked to specific aetiological agents,[12,14,15] but there is no comprehensive study evaluating the differences in outcome and development of laminitis in a large number of diarrhoeic horses with several different aetiologies. Therefore, the objective of this retrospective multicentre study was to determine the aetiological agents associated with acute equine diarrhoea worldwide, their association with outcome of survival and laminitis, and describe the diagnostic approaches used by clinicians for the aetiological diagnosis of diarrhoeic horses. We hypothesised that the diagnostic approaches and detected pathogens of horses with acute diarrhoea vary among referral institutions worldwide and that detection of specific pathogens is associated with the outcome and the development of laminitis.

2 | MATERIALS AND METHODS

Participating institutions were identified by contacting veterinarians via email. The minimum number of horses required to participate was 30 cases admitted to each institution between 2016 and 2020. Inclusion criteria comprised horses >1 year old presenting for acute diarrhoea of <48 h of onset. Horses that developed diarrhoea within the first 24 h after admission were also included, but horses that underwent surgery and developed diarrhoea soon after surgery were excluded. From each institution medical record, demographic data (sex, breed, age), month, season (only for institutions from the Southern and Northern hemispheres, 23.5°–66.5° North and South of Equator, 0°) and year of presentation were registered. In the Northern hemisphere, seasons were classified as winter (December, January, and February), spring (March, April, and May), summer (June, July, and August) and fall (September, October, and November). For the Southern hemisphere, seasons were classified as winter (June, July, and August), spring (September, October, and November), summer (December, January, and February) and fall (March, April, and May). Development of laminitis (yes or no) during hospitalisation and survival to hospital discharge (yes or no) were also recorded.

Information regarding the antemortem testing for common pathogens (i.e., *Salmonella*, *C. difficile*, ECoV and *N. risticii*) were recorded, as well as the type of sample submitted and the tests performed (e.g., polymerase chain reaction [PCR], faecal culture, enzyme-linked immunosorbent assay [ELISA]). *Salmonella* cases were defined as horses with at least one positive result on faecal culture or PCR. *C. difficile* cases were defined as horses with at least one positive result either on ELISA for toxins A and/or B or PCR for *tcdA* and *tcdB* genes. *N. risticii* cases were defined as horses with at least one positive result either on blood or faecal PCR testing or serum indirect fluorescent antibody (IFA) titres for *N. risticii* titres >1:80.[16,17] EcoV cases were defined as horses with at least one positive result on PCR testing for EcoV in a faecal sample. The number of horses diagnosed with sand enterocolitis was also recorded based on clinical and radiographic assessment. Horses that were treated with antimicrobial drugs for a specific clinical diagnosis other than gastrointestinal illness and developed diarrhoea during antimicrobial therapy were considered to have (AAD). Information regarding the detection of *Clostridium perfringens* was not recorded.

2.1 | Data analysis

The normality of the data was assessed using normal probability Q–Q plots and the Kolmogorov–Smirnov test, and data were analysed accordingly. Descriptive statistics included mean, standard deviation (SD), median, and ranges. Categorical variables were compared between groups using X^2 or Fisher's exact tests, while continuous variables were compared with a Student's *t*-test or a Mann–Whitney test. The detection rate of each aetiological agent was calculated as the number of horses positive for an agent over the number of horses tested and was compared between institutions or groups (e.g., laminitis) using a X^2 test. A comparison of the proportion of surviving horses positive for each of the tested aetiological agents was performed using a X^2 or Fisher's exact. Then, crude odds ratios (OR) and 95% confidence intervals (CI) were calculated.

The level of agreement between the first *Salmonella* culture and a PCR to detect *Salmonella* DNA in fresh faeces or faecal samples enrichment in broth for 24 h was assessed using the Kappa coefficient test. In addition, the level of agreement between the ELISA and a PCR test for the detection of *C. difficile* and the level of agreement between PCR testing for *N. risticii* DNA detection in blood or faeces were explored using the Kappa coefficient test. The Kappa agreement

was judged as poor when $0 > k < 0.40$, fair when $0.41 > k < 0.59$, good when $0.60 > k < 0.74$, and excellent when $0.75 > k < 1.0$.[18]

A p-value <0.05 was considered statistically significant. Statistical analyses and figures were performed using statistical software (StataCorp. 2021. Stata Statistical Software: Release 17. StataCorp LLC) and JMP (JMP 16, SAS Institute Inc.).

3 | RESULTS

3.1 | Participating institutions

Fifty-six institutions were contacted and invited to collaborate with the study; 40 agreed to participate, and 16 did not respond. After an initial review of their medical records, 10 institutions concluded they did not have enough cases to contribute. Three indicated that they could not collect the data due to time constraints. One institution submitted a small number of cases with limited information for analysis and was excluded from the study. This left 26 institutions located in 14 different countries (Australia [$n = 4$], Canada [$n = 2$], Chile, Colombia, Denmark, England, Finland, France, Ireland, Italy, Japan, Mexico, Switzerland, and the United States [$n = 9$]) from five different geographic areas (North America, Latin America, Australia, Asia and Europe) with cases presented between 1 January 2016 and 31 December 2020, for analysis. Institutions from North America included Auburn University (AU), University of Prince Edward's Island (UPEI), Iowa State University (ISU), Kansas State University (KSU), Marion duPont Equine Medical Center (MdP), Rood and Riddle Equine Hospital (RREH), The Ohio State University (the OSU), University of Florida (UF), University of Guelph (UG), University of Wisconsin-Madison (UW), Washington State University (WSU). Institutions from Europe included Fethard Equine Hospital (Fethard, Ireland), University of Copenhagen (Copenhagen), University of Helsinki (Helsinki), University of Lyon (Lyon), University of Perugia (Perugia), The Royal Veterinary College (RVC) and University of Zurich (Zurich). Australian institutions were The University of Adelaide (Adelaide), the University of Melbourne (Melbourne), Murdoch University (Murdoch), and the University of Queensland (UQ). Institutions from Latin America included Universidad Austral de Chile (AUCh), Universidad Nacional de Colombia (UNAL) and Universidad Nacional Autonoma de Mexico (UNAM), whereas, from Asia, the Japan Racing Association Ritto Training Center (JRA Ritto) was included.

3.2 | Horses

A total of 1438 horses met the inclusion criteria. Of 1438, 630 (44%) were presented to institutions in North America, 483 (33%) in Europe, 149 (10%) in Latin America, 141 (10%) in Australia and 35 (2%) in Japan. The number and proportions of horses admitted in each institution and detailed information on presenting complaint, time of the year and other epidemiological information is reported elsewhere.[19] This study included 635 (47%) females and 763 (53%) males. The age of the horses ranged between 1 and 35 years (median: 9 years). Thirty-four breeds were represented, with Thoroughbred (283/1438, 20%), Quarter Horses (203/1438, 17%), ponies (140/1438, 10%) and Draught horses (113/1438, 8%) being the most prevalent breeds.

4 | AETIOLOGICAL AGENT INVESTIGATION

An enteropathogen was identified in only 16% (235/1438) of the horses, but not every horse was tested for each pathogen. Table 1 summarises the testing for *Salmonella* spp., EcoV, *C. difficile* and *N. risticii* at each institution.

4.1 | *Salmonella* faecal cultures

The most frequently used method to test for *Salmonella* was a faecal culture, with 626/1438 (44%) horses being tested at least once, of which 67/626 (11%) tested positive. In 391/1438 (27%) cases, one culture was performed, two cultures in 73/1438 (5%), 3 in 122/1437 (8%), 4 in 24/1438 (2%) and 5 in 204/1438 (14%) cases. A high intra- and inter-institutional variability regarding the number of *Salmonella* cultures performed in each case of acute diarrhoea was observed (Figure 1). Auburn and UF institutions performed faecal culture for *Salmonella* at least once in every horse admitted with acute diarrhoea, and Zurich tested 97% (100/103) of the horses using one faecal culture. Regarding serial faecal cultures for *Salmonella* detection at AVC and RVC, 83% (10/12) and 75% (30/40) of the horses had three faecal cultures performed, respectively. In contrast, at UF and Melbourne, 69% (26/38) and 64% (39/61) of the horses had five faecal cultures for *Salmonella*, respectively.

In 204 horses, five faecal cultures were performed 12–24 h apart, with 34 (17%) being positive for *Salmonella*. Of those 34 positive horses, 19/34 (56%) were positive in both the first and second faecal cultures, and 29/34 (85%) were positive after the third faecal culture. After the fourth faecal culture, 31/34 (91%) horses tested positive, and by the fifth faecal culture, all 34/34 (100%) horses tested positive for *Salmonella*.

4.2 | *Salmonella* PCR

A PCR test for *Salmonella* in fresh faeces and faeces after 24 h of enrichment broth was performed in 227/1438 (16%) and 125/1438 (9%) horses, respectively. Twenty-four of 227 (11%) horses tested positive in fresh faeces and 11/168 (7%) in enriched faecal samples. Both PCR in fresh faeces and enrichment media was performed in 125 horses, with three being positive in both tests. However, two horses that were negative for *Salmonella* in fresh faeces were positive in enrichment samples. The institutions that proportionally tested a greater number of horses for *Salmonella* using PCR in faeces were KSU (85%, 18/21), ISU (77%, 23/30), MdP (72%, 23/32), UQ (61%, 22/36), the OSU (55%, 31/56), RREH (51%, 60/117) (Table 2).

TABLE 1 Etiologic testing for pathogens associated with diarrhoea in 1438 horses presented to 26 institutions from North America, Latin America, Europe, and Japan.

Institution [*n*]	*Salmonella* spp.			*C. difficile*	*N. risticii*	EcoV
	Culture	PCR in fresh faeces	PCR after broth enrichment			
	% [horses tested positive/number of horses tested]					
Adelaide [*n* = 24]	17% [3/18]	0% [0/12]	0% [0/12]	0% [0/16]	-	0% [0/12]
Auburn [*n* = 47]	17% [8/47]	-	50% [1/2]	13% [2/15]	0% [0/2]	14% [1/7]
UPEI [*n* = 12]	9% [1/11]	-	-	-	-	33% [1/3]
UACh [*n* = 4]	-	-	-	-	-	-
Copenhagen [*n* = 110]	3% [2/62]	-	-	5% [3/60]	-	15% [3/20]
FETHARD [*n* = 22]	0% [0/4]	-	-	-	-	-
Helsinki [*n* = 156]	0% [0/25]	-	0/1	0% [0/12]	-	25% [1/3]
Iowa [*n* = 30]	8% [2/25]	100% [1/1]	4% [1/22]	0% [0/11]	8% [1/12]	0% [0/9]
JRA/Ritto [*n* = 35]	0% [0/7]	-	-	7% [2/28]	-	-
KSU [*n* = 21]	0% [0/5]	13% [2/13]	6% [1/17]	0% [0/15]	0% [0/14]	14% [2/14]
Lyon [*n* = 37]	20% [2/10]	50% [2/4]	17% [1/7]	25% [1/4]	-	0% [0/5]
MdP [*n* = 32]	4% [1/26]	0% [0/23]	4% [1/23]	8% [2/25]	16% [4/24]	9% [2/21]
Melbourne [*n* = 61]	9% [5/56]	0% [0/7]	0% [0/1]	0% [0/7]	-	0% [0/4]
Murdoch [*n* = 20]	0% [0/2]	-	7% [1/14]	14% [2/14]	-	7% [1/13]
The OSU [*n* = 56]	9% [4/47]	14% [2/14]	18% [3/17]	6% [2/31]	28% [8/29]	0% [0/32]
Perugia [*n* = 15]	100% [3/3]	-	-	-	-	-
RREH [*n* = 117]	56% [9/16]	15% [9/60]	0% [0/1]	7% [5/70]	3% [2/73]	3% [2/68]
RVC [*n* = 40]	14% [5/37]	0% [0/1]	-	0% [0/3]	-	0% [0/1]
UF [*n* = 38]	11% [4/38]	0% [0/7]	14% [1/7]	0% [0/8]	0% [0/6]	18% [2/11]
UG [*n* = 191]	0.7% [1/144]	0% [0/6]	0% [0/1]	4% [3/77]	31% [15/48]	9% [3/33]
UNAL [*n* = 31]	26% [5/19]	-	-	-	-	-
UNAM [*n* = 94]	-	66% [2/3]	-	-	-	-
UQ [*n* = 36]	22% [6/27]	23% [5/22]	0% [0/1]	0% [0/20]	-	0% [0/20]
UW [*n* = 44]	8% [3/37]	0% [0/20]	0% [0/12]	0% [0/20]	24% [5/21]	0% [0/20]
WSU [*n* = 42]	4% [1/23]	0% [0/30]	3% [1/29]	11% [4/37]	0% [0/32]	6% [2/32]
Zurich [*n* = 103]	2% [2/102]	-	-	0.9% [1/102]	-	-

Adelaide, The University of Adelaide; AU, Auburn University; AUCh, Universidad Austral de Chile; Copenhagen, University of Copenhagen; FETHARD, FETHARD Equine Hospital; Helsinki, University of Helsinki; ISU, Iowa State University; JRA Ritto, Japan Racing Association Ritto Training Center; KSU, Kansas State University; Lyon, University of Lyon; MdP, Marion duPont Scott Equine Medical Center; Melbourne, University of Melbourne; Murdoch, Murdoch University; Perugia, University of Perugia; RREH, Rood and Riddle Equine Hospital; RVC, The Royal Veterinary College; The OSU, The Ohio State University; UPEI, University of Prince Edward's Island; UF, University of Florida; UG, University of Guelph; UNAL, Universidad Nacional de Colombia; UNAM, Universidad Nacional Autonoma de Mexico; UQ, University of Queensland; UW, University of Wisconsin-Madison; WSU, Washington State University; Zurich, University of Zurich.

[Correction added on 6 December 2023, after first online publication: Table 1 has been corrected in this version.]

Overall, 81/633 (13%) of horses tested positive for *Salmonella* using faecal culture or PCR in fresh faeces or after enrichment broth. *Salmonella* was reported in all institutions except Fethard, UNAM and UACh; however, UACh and UNAM did not test any horses for *Salmonella*, and Fethard only tested 19% (4/22, Table 1). The institutions with >10% of tested horses for *Salmonella*, using faecal culture or PCR assays, that were positive included UNAL (26%, 5/19), UQ (22%, 8/36), Perugia (20%, 3/15), Lyon 20% (2/10), Auburn (19%, 9/47), RVC (12.5%, 5/40), the OSU (11%, 6/56) and UF (10.5%, 4/38, Table 1). There was no effect of season on the detection rates of *Salmonella*, with 8.3% (12/144) of the horses tested in the fall being positive, 16% (21/134) in the spring, 13% (21/161) in summer, and 12% (20/173) in winter ($p = 0.28$).

4.3 | Agreement between the faecal culture and PCR testing for the detection of *Salmonella*

In 145 horses, the results of the first faecal culture and a PCR test from fresh faeces were available. Eleven horses were positive in the faecal culture, and 10 were also positive in the PCR test, while 8 were positive on the PCR test and negative on the faecal culture. The Kappa statistic was 0.65 (95%CI: 0.45–0.86, $p < 0.001$).

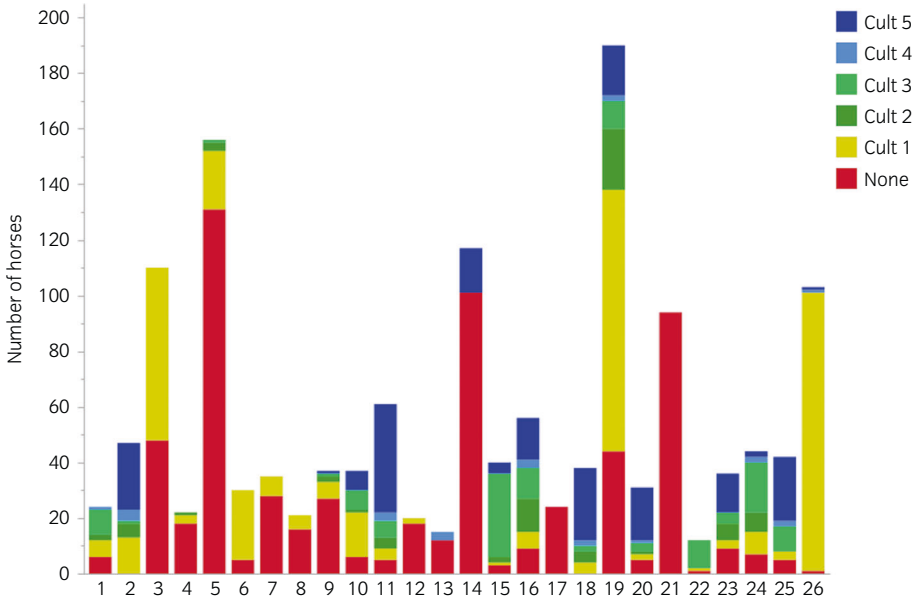

FIGURE 1 The number of bacteriological faecal cultures performed to detect *Salmonella* spp. in 1438 diarrhoeic horses admitted at 26 different institutions worldwide. Cult, culture.

In 125 horses, the results of the first faecal culture and PCR testing in faeces after 24 h of culture enrichment were available. Five cultures were positive, whereas nine samples were PCR positive. All positive culture samples were also positive on the PCR test, whereas the remaining four PCR test-positive samples were negative on the faecal culture. The Kappa statistic was 0.69 (95%CI: 0.42–0.97, $p < 0.001$).

4.4 | *Clostridiodes difficile* testing

Clostridioides difficile was the second most frequently identified aetiological agent, with 578/1438 (40%) horses being tested. Twenty (77%) institutions tested at least one horse for *C. difficile*, while 6 (23%) institutions did not test any of the horses (Fethard, UPEI, UACh, Perugia, UNAL and UNAM). The institutions that tested a greater proportion of horses for *C. difficile* were Zurich (99%, 102/103), WSU (88%, 37/42), MdP (78%, 25/32), KSU (71%, 15/21), Murdoch (70%, 14/20), JRA/Ritto (80%, 28/35), and RREH (60%, 70/117) (Table 1).

Clostridioides difficile was detected in 5% (27/578) of the tested horses. The proportion of horses positive for *C. difficile* varied from 0% to 25%, with the highest proportion being positive at Lyon (Table 2). A total of 20% (286/1438) of the horses were tested for *C. difficile* only by ELISA for toxins A/B, of which 5.6% (16/286) were positive, and 17% (249/1438) of horses were tested for *C. difficile* only by PCR for *tcdA* and *tcdB* genes, of which 5% (12/249) were positive.

TABLE 2 Association of survival and development of laminitis with pathogen detection and identification of sand in the colon of horses with acute diarrhoea.

	Survival		Laminitis	
	Yes	Not	Yes	Not
Salmonella				
Positive	61 [75%]	20 [25%]	5 [6%]	75 [94%]
Negative	423 [76%]	129 [24%]	39 [7%]	508 [93%]
C. difficile[a]				
Positive	15 [55%]	12 [44%]	4 [18%]	23 [85%]
Negative	425 [77%]	126 [23%]	41 [7%]	508 [93%]
ECoV				
Positive	30 [81%]	7 [19%]	2 [5%]	35 [95%]
Negative	266 [77%]	78 [23%]	32 [8%]	350 [92%]
N. risticii				
Positive	27 [77%]	8 [23%]	8 [23%]	27 [77%]
Negative	178 [78%]	49 [22%]	22 [9%]	205 [91%]
Sand				
Positive	55 [83%]	11 [17%]	2 [3%]	64 [97%]
Negative	1038 [75%]	334 [25%]	98 [7%]	1253 [93%]

Abbreviations: *C. difficile*, *Clostridiodes difficile*; ECoV, equine coronavirus; *N. risticii*, *Neoricketsia risticii*.

[a]*C. difficile*-positive horses had greater odds of not surviving than *C. difficile*-negative horses (Odd Ratio [OR]: 2.69, 95%CI: 1.23–5.91; $p = 0.016$).

4.5 | Agreement between ELISA for *C. difficile* toxins A/B and PCR for toxins A/B genes

A total of 42 horses were tested for *C. difficile* toxins A/B by ELISA and *C. difficile* by PCR for *tcdA* and *tcdB* genes, of which 7% (3/42) were positive in both tests and 19% (8/42) were positive in the ELISA test but negative in the PCR test. The Kappa statistic for ELISA for toxins A/B and PCR for *tcdA* and *tcdB* genes for *C. difficile* diagnosis yielded fair agreement ($k = 0.48$, 95%CI: 0.13–0.84; $p < 0.001$).

4.6 | *Neorickettsia risticii*

Neorickettsia risticii was the third most frequently investigated aetiological agent. A total of 262/1438 (35%) horses were tested either by a PCR test in faeces and/or blood or using an IFA test (only at the OSU). However, when only horses from North American institutions (n = 427) were included in the analysis, 262/630 (42%) were tested for *N. risticii*. None of the European, Latin American or Japanese institutions tested any of the horses for *N. risticii*. All North American institutions except UPEI tested at least one horse for *N. risticii*. The institutions testing a greater proportion of horses for *N. risticii* were MdP (75%, 24/32), KSU (67%, 14/21), RREH (62%, 73/117) and the OSU (52%, 29/56, Table 2).

A total of 35/262 (13%) horses were positive for *N. risticii*. Thirty-seven horses were tested using the IFA test, with a total of 7/37 (19%) horses being positive; 76 horses were tested using a PCR test in blood, with 24% (18/76) being positive; 258 horses were tested for *N. risticii* by PCR in faeces with 10% (26/258) being positive. The proportion of horses positive for *N. risticii* varied from 0% to 31%, with the highest proportion being positive at UG.

4.7 | Agreement between blood and faecal PCR assay for *Neoricketsia risticii*

In 67 cases, blood and faecal PCR tests were performed for *N. risticii* DNA detection, with 14 positive blood and faecal samples. Only one horse that tested positive for *N. risticii* DNA in faeces tested negative in blood. The Kappa statistic for blood and faecal PCR assay for *N. risticii* DNA detection yielded excellent agreement ($k = 0.95$, 95% CI: 0.87–1; $p < 0.001$).

4.8 | Equine coronavirus

Equine coronavirus was the least frequently tested pathogen (23%; 331/1438), with 6% (26/331) horses being positive. The five institutions testing the greatest proportion of horses for EcoV were MdP (72%, 23/32), KSU (67%,14/21), RREH (58%, 73/117) and the OSU (57%, 32/56). EcoV was detected in 8/11 (72%) institutions from North America (Auburn, UPEI, KSU, MdP, RREH, UF, UG and WSU), Europe (Copenhagen, Helsinki), and Australia (Murdoch); however, neither Latin American nor Japanese institutions tested for ECoV. The proportion of positive cases varied between institutions from 0% to 18% (Table 1).

4.9 | Antimicrobial-associated diarrhoea

In total, 8.3% (120/1438) of the horses were diagnosed with AAD. Body systems that were being treated with antimicrobial drugs before developing diarrhoea included the integumentary (29%, 35/120), musculoskeletal (27%, 32/120), respiratory (25%, 31/120), urogenital (3%, 4/120) and ophthalmic (3%, 4/120). Antimicrobial therapy included monotherapy with trimethoprim/sulfonamide (20%, 24/120), doxycycline (7.5%, 9/120) or ceftiofur (4%, 5/120) and the combination of penicillin and gentamicin (15%, 19/120).

Of the 120 horses with AAD, 54 were tested for *C. difficile* via ELISA or PCR for *tcdA* and *tcdB* genes, and 19% (10/54) were positive; 72/120 animals were tested for *Salmonella*, of which 11% (8/72) were positive; 30/120 were tested for *N. risticii*, and 10% (3/30) were positive; and 58/120 horses were tested for ECoV, and all of them were negative.

4.10 | Coinfections

Coinfections were reported in seven horses, with the detection of *Salmonella* and ECoV in two horses, *Salmonella* and *C. difficile* in three horses, *C. difficile* and *N. risticii* in one horse, and *C. difficile* and ECoV in one horse.

4.11 | Sand-associated diarrhoea

Sixty-six (5%) of the 1438 horses with acute diarrhoea were diagnosed with sand-associated diarrhoea. This disease was reported in six institutions from North America (UW, UF, ISU, KSU, MdP and WSU), two from Australia (Adelaide and Murdoch) and two from Europe (Helsinki and Copenhagen). The institutions with the highest proportion of horses diagnosed with sand-associated diarrhoea were UF (26%, 9/38) and Helsinki (23%, 36/156). Of the 66 horses diagnosed with sand-associated diarrhoea, 13/66 were tested for EcoV, and 3/13 (23%) were positive; 24/66 were tested for *Salmonella* at least using one faecal culture or via PCR in fresh faeces or after 24 h of enrichment broth, 7/66 for *C. difficile* via ELISA or PCR test and 5 for *N. risticii* (all via PCR in faeces). All horses tested negative for *Salmonella*, *C. difficile* and *N. risticii*.

5 | SURVIVAL TO HOSPITAL DISCHARGE

The overall survival proportion for diarrhoeic horses admitted to 26 institutions was 76% (1093/1438). In total, 306 horses were euthanised for poor prognosis (176/208, 89%) or economic reasons (22/345, 11%).

The survival proportion of horses in which sand accumulation or a pathogen was associated with diarrhoea is presented in Table 2. The proportion of *C. difficile*-positive horses that died or were euthanised was greater (12/27, 44%) than *C. difficile*-negative horses (126/551, 23%). *C. difficile*-positive horses had greater odds of not surviving than *C. difficile*-negative horses (OR: 2.69, 95%CI: 1.23–5.91; $p = 0.02$). There was no other association between the detection of any pathogen and the survival of horses with diarrhoea.

6 | AETIOLOGICAL AGENTS, SAND-ASSOCIATED DIARRHOEA AND LAMINITIS

Out of 100 horses that develop laminitis during hospitalisation, 85 (85%) were presented to institutions from the Northern and Southern hemispheres. In total, 46% (39/85) of the horses that developed laminitis were admitted during the summer, and this was statistically higher than in winter (18%, 15/85), spring (18%, 15/85) and fall (19%, 16/85) ($p < 0.01$, for all comparisons).

There was no association between detecting any pathogen and developing laminitis in horses with acute diarrhoea (Table 2). However, when only horses from North America were analysed, among the horses tested for *N. risticii*, the proportion of positive horses that developed laminitis was higher (23%, 8/35) than those that tested negative (9%, 22/227). Specifically, horses that were positive for *N. risticii* had greater odds of developing laminitis than negative horses (OR: 2.76, 95%CI: 1.12–6.81; $p = 0.03$). No other differences were identified in the different geographic areas.

7 | DISCUSSION

This study documented limited diagnostic investigation in cases of acute equine diarrhoea. Aetiological testing was primarily limited to a single faecal culture for *Salmonella*, with only 44% of the horses being tested for this pathogen at least once. *N. risticii*, *C. difficile*, and ECoV were also investigated in horses enrolled in this study, with only 42%, 40% (only North America), and 29% being tested for each pathogen, respectively. Reasons for the lack of pathogen testing are not readily apparent in this study but may include the epidemiology of the disorders (e.g., *N. risticii* is a pathogen detected in North and South America but not in Europe, Australia, or Japan), awareness of the pathogen, and test availability in the local or regional laboratories (e.g., some tests were unavailable in Latin American and certain countries in Europe). Additionally, economic constraints and owner and clinicians' attitudes and perceptions regarding aetiological agent identification might play a role as the testing can be expensive, confusing (e.g., detection of *C. perfringens* cpa toxin gene as the virulence of this toxin is negligible),[20] and is unlikely to alter the treatment (except in cases of *N. risticii*) or prognosis of the horses.[2] Differences in clinicians' attitudes and perceptions regarding aetiological agent identification can also explain in part the inter- and intra-institutional differences in the testing approach. For example, the current recommendation for detecting *Salmonella* in horses with diarrhoea is testing multiple samples (e.g., 3–5) using enriched methods to increase the overall sensitivity of the test.[1,7] These clinical practice recommendations have been developed to guide clinicians and client decision-making, reduce variability in clinical practice and minimise intuitive and anecdotal decision-making. However, in our study, some horses were not tested for *Salmonella* within the same institutions, whereas others were tested using 1, 3, or 5 faecal cultures. These findings demonstrated that developing and publishing guidelines does not translate into their implementation in the clinical practice.[21] Reasons for this include a perception by clinicians that guidelines can restrict their autonomy and impede their ability to tailor the management of the case to the horse's and client's individual needs and preferences, as demonstrated in the human medicine.[22]

The Havemeyer workshop on acute colitis held in 2019 concluded that an '*… aetiological agent is not identified in >50% of the cases of acute colitis*'.[1] In our study, an enteropathogen was only detected in 16% (235/1438) of the horses. However, we showed limited and inconsistent antemortem aetiological agent testing in many cases of acute diarrhoea. Therefore, implementing in-house equine diarrhoea pathogen panels using PCR technology has been proposed to improve and accelerate pathogen detection, discovering co-infections and identifying pathogens that can be missed with traditional methodologies.[22] However, the cost–benefit of implementing those equine diarrhoea pathogen panels must be investigated. A potential reason for the lack of pathogen detection in diarrhoeic horses is the existence of yet unidentified pathogens. Recently, NetF-producing toxin *C. perfringens*,[23,24] *Paeniclostridium sordellii*[25,26] and *Clostridium innocuum*[27] have been proposed as a potential agents causing diarrhoea in horses. Also, metagenomic studies have consistently identified an increase in the genus *Fusobacteria* in diarrhoeic horses.[28] Therefore, additional studies are needed to uncover the role of these bacteria in the pathogenesis of equine diarrhoea.

The overall proportion of horses positive for *Salmonella* was 13% and fell within the shedding prevalence reported in hospitalised horses worldwide, ranging between 3% and 23%.[29] As expected, major differences in detection rates were observed among institutions, ranging between 0% and 22%. The number and type of tests performed, and the actual prevalence of Salmonella in some geographic regions can explain the differences. The marked geographical and seasonal differences regarding the annual incidence of Salmonellosis are documented with higher detection rates in the summer in southern United States compared to other regions.[30–33] This agrees with our results showing that geographic areas with warmer and wetter climates (e.g., Auburn and Florida and Colombia) had the highest detection rates of *Salmonella*.

Our results indicated that the faecal detection rates of *Salmonella* increased with each additional sample submitted for culture. This is in agreement with previous reports.[6] Although the culture of five consecutive faecal samples remains the standard protocol for clinical diagnosis of equine salmonellosis, the submission of faecal samples serially collected for culture was highly variable among institutions. Therefore, the exact number of *Salmonella*-associated diarrhoea was likely underestimated in our study. PCR assays are highly sensitive and specific methods to detect *Salmonella* in faecal samples, particularly after pre-incubation in enrichment broth.[34] In this study, the sensitivity of the PCR was not assessed, but we found a good agreement between the first faecal culture and a PCR assay in fresh faeces or faeces pre-incubated in broth. The diagnostic value of serial PCR tests for detecting *Salmonella* in diarrhoeic horses warrants further investigation.

Neorickettsia risticii is a major cause of systemic illness in horses in North America that requires antimicrobial therapy. Therefore, it

was expected to be one of the most tested enteropathogens, with 61% of the horses presented to North American institutions being tested for this agent. The detection rate of *N. risticii* varied between 0% and 31%, with the highest rates reported in Ontario, Canada and institutions located in the Midwest region of the United States, especially during the summer and fall seasons. This finding agrees with previous reports from single-centre studies[35] or from analysis of samples submitted to a diagnostic laboratory to detect enteropathogens.[12,14]

Detection of DNA of *N. risticii* in faecal or blood samples is recommended for the diagnosis of Neorickettiosis (Potomac horse fever).[6] In our study, the level of agreement between blood and faecal PCR assays for detection of DNA of *N. risticii* was excellent. Although this finding could indicate that either sample can be submitted for DNA detection of *N. risticii*, a difference in the time and duration of molecular detection of *N. risticii* in blood versus faecal samples has been demonstrated in experimentally infected horses with blood samples testing positive earlier than faeces.[36] In addition, the agreement between blood and faecal PCR assay for detecting *N. risticii* DNA varies between laboratories from poor to good.[36] Thus, submitting both samples appears to be the most appropriate method for testing *N. risticii*. The IFA test is a primary method used to diagnose *N. risticii*. However, a high rate of false-positive results has been reported for this test.[37] Recently, *Neorickettsia findlayensis*, another Neorickettsial species capable of causing disease, was isolated from two horses in Canada.[38] The prevalence of this new *Neorickettsia* spp. is unknown; however, including this new species in newly designed PCR faecal panels for equine diarrhoea might be prudent. Alternatively, laboratories could offer PCR testing to detect *Neorickettisa* spp., rather than species-specific assays.

Laminitis is a common complication reported in horses suffering from *N. risticii* infection.[7,14,15] Therefore, it was not surprising that horses that tested positive for this pathogen were more likely to develop laminitis. Despite extensive research on laminitis, the reason for the increased occurrence of laminitis in *N. risticii*-positive horses is currently unknown.

Clostridiodes difficile has been reported to be the most common aetiological agent detected in horses that succumb to colitis.[1,3] *C. difficile* was the second most frequently investigated aetiological agent with 40% of cases tested in 77% of the institutions. The reported prevalence of *C. difficile*-associated disease (CDAD) in adult horses with diarrhoea varies from 0% to 40%.[4,9,13,39–43] In our study, the proportion of horses positive for *C. difficile* was similar to those reported varying from 0% to 25% depending on the institution.

Direct *C. difficile* toxin detection (ELISA, cytotoxicity assay) is preferred over detecting toxin genes as the latter could identify carrier states.[1] In our study, 50% of horses tested for *C. difficile* were tested using an ELISA assay, 43% were tested by toxigenic culture (culture followed by toxin gene PCR), and 7% were tested with both methods. The reported sensitivity and specificity of the ELISAs are highly variable from 78% to 90%.[44–46] The agreement between PCR for *tcdA* and *tcdB* genes and ELISA for detecting toxins A and B in our study was only fair. Furthermore, 8/42 horses tested positive on the ELISA but were negative on the toxigenic culture; this is likely due to the difficulty of culturing *C. difficile* and further highlights that toxin detection may be clinically more relevant over toxin gene detection by PCR.[1]

Healthy horses can harbour and shed *C. difficile*; however, the number of carrier horses is relatively low, with most studies reporting a prevalence of toxigenic *C. difficile* without toxin production in healthy adult horses of <5% (range 0%–25%).[5,40,47–50] Therefore, most likely, all horses in this study were CDAD cases, as 50% of our cases were diagnosed based on toxin detection. The cumulative sampling over the course of 3 days in horses with colic increased recovery of *C. difficile* by 9%,[4] likely as shedding could be intermittent, which has been found in healthy horses.[49] In our study, only one faecal sample was tested per horse in all institutions, which could have resulted in horses with CDAD being missed.

Antimicrobial administration is a risk factor for the development of CDAD in horses.[9,40,43,51] Correspondingly, in our multi-centre study, *C. difficile* was the most frequently detected pathogen in horses with AAD (19%). Mortality of CDAD in horses varies greatly between 0% and 83%.[9,41,52] In our multicentre study, horses with CDAD were 2.7 more likely to die or be euthanised than *C. difficile* negative cases. This agrees with previous single-centre studies showing a higher fatality rate in horses with CDAD than those with colitis but negative to *C. difficile*.[9,11]

ECoV infection in adult horses has been reported in Japan,[53,54] North America[53] and Europe.[8,10,14,55–60] This is in accordance with our study, where cases were identified in all participating institutions in Europe, North America and Australia that tested for ECoV, with an overall detection rate of 6%. However, as ECoV is an emerging disease, testing for ECoV was not performed by all participating institutions, with only 23% of cases tested in total. This detection rate was similar to the previously reported in horses with clinical signs presented to primary care veterinarians and from summary data reported by US diagnostic laboratories, which range between 2% and 7%.[61,62] However, 70%–80% of horses with ECoV infection display signs of unspecific viral disease without gastrointestinal signs,[35,53,63] thus the detection rate of ECoV in our study could have underestimated the level of infection as only horses with acute diarrhoea were included.

The molecular detection of ECoV in the faeces of horses with gastrointestinal signs does not necessarily prove that the virus is involved in the disease process, especially for foals where ECoV has been detected in healthy and diseased foals.[64,65] However, a strong association between clinical status and PCR detection of EcoV is reported in adult horses.[62] Therefore, it is likely that most, if not all, horses with a positive ECoV PCR test in our study showed clinical signs due to ECoV infection. This is further supported by a low rate of co-infections with other pathogens, which is consistent with previous studies.[66,67] In all institutions, only a single faecal sample was tested for ECoV. Some horses can test negative on initial sampling but become positive 24–48 h after admission.[66] Possible explanations include initial stasis of the gastrointestinal tract due to infection with little viral DNA present in faeces. Faecal shedding in experimentally infected horses starts 3–4 days post-infection and peaks 3–4 days after the development of clinical signs.[67,68] Evaluation of multiple or

pooled samples might also be a necessary strategy for detecting ECoV infection, similar to the current recommendation for *Salmonella* testing.

Sand accumulation is a well-known cause of gastrointestinal disease in certain parts of the world with loose sandy soil, such as California, Florida, and the southwestern United States,[69,70] Finland,[71] Denmark[72] and anecdotally in Australia. In this multi-centre study, 5% of horses were diagnosed with sand-associated diarrhoea; however, infectious agents were not thoroughly ruled out, with only 45/66 horses being tested for a single aetiological agent. Institutions with a prevalence of >20% were located in Florida and Finland, which have reported a high prevalence of sand enteropathy in the past.[69,71]

Our results are limited by the study's retrospective design, which prevents data collection and sample processing from being standardised within and between institutions which could have resulted in information bias. This limitation could result in missing data for several variables especially those regarding pathogen testing and detection. The study collected data from diarrhoeic horses presented to referral institutions could bias the study towards sicker animals. Therefore, it is important to be cautious when applying these results to a different population of horses. The number of cases provided varied significantly between institutions, but it likely reflects the institution's population. Nonetheless, this study provided a comprehensive analysis of the epidemiology of sand-associated diarrhoea and the pathogens detected in horses with acute diarrhoea. It offered an overview of the diagnostic approaches for equine diarrhoea used by clinicians in different institutions worldwide.

AUTHOR CONTRIBUTIONS

D. Gomez, D. Renaud and R. Toribio contributed to the study design, data collection, analysis and interpretation, and manuscript preparation. B. Dunkel, L. Arroyo, A. Schoster, J. Kopper and D. Byrne contributed to the data collection, and interpretation and manuscript preparation. A. Mykkanen, W. Gilsenan, T. Pihl, G. Lopez-Navarro, B. Tennent-Brown, L. Hostnik, M. Mora-Pereira, F. Marques, J. Gold, S. DeNotta, I. Desjardins, A. Stewart, T. Kuroda, E. Schaefer, O. Oliver-Espinosa, G. Ferlini-Agne, B. Uberti, P. Veiras, K. Delph Miller, R. Gialleti and E. John contributed to data collection and manuscript preparation. All authors approved the final version of the manuscript. D. Gomez had full access to all the study data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request: Open sharing exemption granted by editor for this descriptive retrospective clinical report.

ETHICAL ANIMAL RESEARCH

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INFORMED CONSENT

Explicit owner consent for animals' inclusion in the study was not stated.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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