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Salmonella spp. in Horses with Diarrhea - Clinical, Laboratory and Microbiological Aspects

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ABSTRACT

***Background*:** Salmonellosis is an important cause of diarrhea in horses with zoonotic implications. Diagnosis depends on the isolation of the agent through serial cultures or molecular techniques. The aim of this retrospective study was to describe the clinical, laboratory, and microbiological aspects of salmonellosis in horses presenting diarrhea referred to a veterinary teaching hospital.

***Materials, Methods & Results*:** Horses presenting signs of diarrhea treated between January 2009 and December 2019, from which *Salmonella* spp. were isolated from feces, were included in this study. Epidemiological data, clinical and laboratory findings (hematology, serum biochemistry, and blood gas analysis), and *in vitro* antimicrobial resistance profiles were analyzed. During this period, 78 of the admitted horses presented diarrhea, and *Salmonella* spp. were isolated from 28% (22/78) of them. Among these patients, 41% (9/22) were younger than 3 months, 27% (6/22) were between 6 and 12 months, 14% (3/22) were between 2 and 7 years, and 18% (4/22) were older than 9 years. Clinical signs included tachycardia, tachypnea, mucous membrane congestion and liquid feces. Clinicopathological tests revealed hyperfibrinogenemia, increased serum gamma-glutamyl transferase serum activity and metabolic acidosis. In addition to feces, *Salmonella* spp. were isolated from 10% (2/22) and 5% (1/22) of lung and intestinal tissues and blood, respectively, from horses that did not survive. The isolates were resistant to sulfamethoxazole/trimethoprim (4/20; 20%) and gentamicin (3/20; 15%), along with partially resistant strains (5/20; 25%) and strains resistant (2/20; 10%) to ceftiofur. The mortality rate in the study population was 59%.

***Discussion*:** The study highlights the severe impact of equine salmonellosis, particularly in cases complicated by septic shock, which contributed to a high mortality rate and limited the assessment of hospitalization duration and prognosis. The confirmed etiology of diarrhea was based on the isolation of *Salmonella* spp. through fecal, tissue, and blood cultures, with systemic dissemination evident in some cases. Serial fecal culture remains the diagnostic gold standard, although a single positive sample can suffice in horses with gastrointestinal symptoms. The antimicrobial resistance profile revealed significant resistance to sulfamethoxazole/trimethoprim, gentamicin, and ceftiofur. Notably, some isolates were partially sensitive (25%) or resistant (10%) to ceftiofur, reflecting concerns about the future efficacy of cephalosporins due to their widespread use in veterinary medicine. However, the absence of multidrug-resistant strains in this study contrasts with findings from other species and age groups, suggesting that antimicrobial use practices and regulatory differences may influence resistance patterns. Necropsy findings were consistent with equine salmonellosis and bacterial enterocolitis, indicating loss of intestinal barrier integrity, bacterial dissemination, and sepsis development. These findings underline the importance of early diagnosis, effective antimicrobial stewardship, and a better understanding of resistance mechanisms to improve outcomes in equine salmonellosis cases. This study confirms that salmonellosis is a prevalent cause of enterocolitis in horses and is responsible for a high mortality rate. We emphasize the importance of evaluating the antimicrobial susceptibility profile in all cases.

Keywords: equines, diarrhea, nosocomial infection, antimicrobial susceptibility, sepsis, colitis.

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INTRODUCTION

Salmonellosis, caused by bacteria of the genus *Salmonella*, affects the gastrointestinal tract of domestic and wild animals, leading to significant health impacts across species [1,35,40,42,56]. As a major zoonotic disease with substantial economic and public health implications, its control requires a comprehensive One Health approach [14].

In horses, *Salmonella* spp. infection can be the primary etiology of diarrhea [2,6,39]. In this context, *Salmonella* spp. infections can manifest as acute or chronic enterocolitis with intermittent diarrhea, alongside potential extraenteric signs such as abortion, fever, anorexia, and lethargy, even without marked gastrointestinal involvement [25,33]. In foals, often present as sepsis or acute enterocolitis, featuring fever, diarrhea, lethargy, and hypovolemic shock [25,57].

Antimicrobial treatment should ideally be based on *in vitro* susceptibility testing, but it sometimes needs to be initiated beforehand due to the time required to obtain the results [4,5]. In horses with sepsis, broad-spectrum antimicrobial drugs are preferable [26,29,31,47,57]. This study aims to characterize the clinical manifestations, laboratory findings, and antimicrobial resistance profiles associated with salmonellosis in horses presenting with diarrhea.

MATERIALS AND METHODS

Data collection

A retrospective study was conducted at the Large Animal Internal Medicine Service of the School of Veterinary Medicine and Animal Science at the São Paulo State University (UNESP), Botucatu campus, São Paulo, Brazil (22°53'09" S and 48°26'42" W). Medical records from January 2009 to December 2019 were reviewed. Horses with gastrointestinal signs (abdominal discomfort and/or intestinal hypermotility) and diarrhea, with the isolation of *Salmonella* spp., were included in the study. The following signalment were assessed: breed, sex, age, and outcome (discharge or death). Clinical examination at the time of admission included analysis of heart and respiratory rates, rectal temperature, mucous membrane color, fecal appearance, intestinal motility, dehydration scores, and appetite. Antimicrobial therapy, analgesics, and anti-inflammatory drugs were described for the therapeutic procedures adopted.

Clinicopathological exams.

A complete blood count, serum biochemistry and whole-blood clotting time (WBCT) were performed in the clinical pathology laboratory of the veterinary teaching hospital following their standard operational protocols. Venous blood gas analysis was performed by radiometer and was obtained SID_3 (Strong Ion Difference) according to formula [SID_3=(Na+K)−Cl] [7].

In addition to the hematological variables for the foals included in the study, the neutrophil-lymphocyte ratio (NLR) [45] was calculated, and the animals were divided into 2 groups based on the outcome. They were also classified using a sepsis score described previously [4,9].

Microbiological cultures for Salmonella *spp.*

The samples were transferred to sterile test tubes containing 10 mL of tetrathionate broth and incubated at 37°C for 12 h for selected isolation of *Salmonella* spp. Notably, in some animals, more than one fecal sample was collected. The samples were subsequently cultured on *Salmonella-Shigella* agar (SS)[1] and kept under aerobic conditions at 37°C for 24-48 h. Colonies with a morphology suggestive of the *Salmonella* genus (colonies with a diameter of 1-2 mm with blackened centers) after 24 h of incubation were subjected to biochemical characterization, including indole production, citrate utilization, lysine degradation, urease production, motility and utilization of glucose, lactose, and citrate.

In vitro antimicrobial susceptibility tests (AST) were conducted through the disk diffusion technique, and the interpretation of the results was based on the Clinical and Laboratory Standards Institute for Enterobacteriaceae [8]. Seventeen antimicrobials[2] from 6 classes were tested: 1)- Aminoglycosides (amikacin AMK: 30 µg and gentamicin GEN: 10 µg); 2)- Beta-lactams and derivates (amoxicillin AMX: 25 µg, ampicillin AMP: 10 µg, cephalexin LEX: 30 µg, ceftiofur CFUR: 30 µg and ceftriaxone CTR: 30 µg); 3)- Fluoroquinolones (ciprofloxacin CIP: 5 µg, enrofloxacin ENO: 5 µg, levofloxacin LVX: 5 µg marbofloxacin MBF: 5 µg, norfloxacin NOR: 5 µg); 4)- Macrolides (azithromycin AZM: 30 µg); 5)- Sulfonamides (sulfamethoxazole/trimethoprim STX: 1.25/23.75 µg), and 6)- Tetracyclines (tetracycline TET: 30 µg). *Salmonella* strains that exhibited resistance to 3 or more classes of antimicrobials were considered multidrug-resistant isolates [31].

Histopathological examination.

Macroscopic postmortem findings and histopathological evaluations of intestinal segments stained with H&E were analyzed.

Statistical analysis.

A statistical analysis of the clinical signs and laboratory test results was performed. These results were analyzed for distribution via the D'Agostino-Pearson normality test and the Shapiro-Wilk test (GraphPad Prism 5 for Windows - GraphPad Software). The data analyzed descriptively are reported as averages and standard deviations if normally distributed and as medians and ranges if not normally distributed.

RESULTS

Study population

During the study period, a total of 823 horses were referred to the hospital, and their medical records were evaluated. Among these, 78 were referred to presenting diarrhea. *Salmonella* spp. was isolated from the fecal, blood, and/or organ samples of 28% (22/78) of the diarrheic horses. In total, 64% (14/22) were male, and 36% (8/22) were female. The median age of the diarrheic horses from which *Salmonella* spp. was isolated was 3.4 ± 6 years. Among these horses: 41% (9/22) were younger than 3 months; 27% (6/22) were between 6 and 12 months; 14% (3/22) were between 2 and 7 years, and 18% (4/22) were older than 9 years. The breed distribution was as follows: 55% (12/22) American Quarter Horse; 32% (7/22) Mangalarga; 5% (1/22) Thoroughbred; 5% (1/22) American Paint Horse and 5% (1/22) mixed breed.

Clinical signs

During inspection of the feces, 73% (16/22) presented liquid diarrhea with a foul odor, 23% (6/22) had pasty diarrhea, and 4% (1/22) presented fluctuations in fecal characteristics (liquid to normal). On admission, the horses presented a mean heart rate of 74 ±31 beats per minute. Tachycardia was detected in 72% (16/22) of the horses (more than 40 beats per minute). The mean respiratory rate was 38 ± 20 breaths per minute, and 23% (5/22) of the horses presented an elevated respiratory rate on admission (more than 16 movements per minute). Intestinal hypermotility was observed in 55% (12/22) of the horses, and the mean rectal temperature was 37.7±1.17°C, with 36% (8/22) of the horses presenting fever (rectal temperature > 38.5°C). In terms of mucous membrane color: 32% (7/22) were congested; 4% (1/22) were icteric, and 4% (1/22) were cyanotic. Dehydration was observed in 96% (21/22) of the horses, with 5% dehydration detected in 36% (8/22), 7.5% in 32% (7/22), and 10% in 27% (6/22) of the horses. With respect to appetite, upon admission, whereas 37% (8/22) had hyporexia or anorexia.

Laboratory findings

A complete blood count was conducted on 21 of the horses included in the study. The primary hematological abnormalities observed included: hyperfibrinogenemia 81% (17/21); neutrophilia 43% (9/21); anemia 38% (8/21); leukocytosis 28% (6/21); leukopenia 24% (5/21); neutropenia 24% (5/21); monocytosis 19% (4/21), and the presence of band neutrophils 4% (1/21). Table 1 provides individual results for each horse, along with the means, median and standard deviation.

Serum biochemistry was performed on 15 of the 22 horses. The findings included: elevated levels of globulin 82% (9/11); urea 73% (11/15); gamma-glutamyl transferase (GGT) 71% (10/14); aspartate aminotransferase (AST) 21% (3/14); creatinine 20% (3/15); reduction in albumin 46% (6/13), and total plasma protein (TPP) 25% (2/8). Individual results for each horse, along with the means, median and standard deviation, are detailed in Table 2.

Blood gas analysis was performed on 6 horses (6/22). The primary abnormality observed was metabolic acidosis, with a reduction in HCO_{3-} 67% (4/6) and an increase in the anion gap 67% (4/6) [Table 3]. All foals included (2, 3, 4, 5, 6, 7, 8 and 9) in the study were classified as non-septic.

Microbiological culture from animals

Salmonella spp. was isolated from the fecal samples of 86% (19/22) of the animals included in this study. The pathogen was isolated from the blood culture of a single animal (horse 21), using a sample collected at the time of discharge. In the remaining cases (horses 2 and 9), the agent was isolated from organ fragments collected during necropsy (lung and intestine) 9% (2/22 - horses 2 and 9). The results of the *in vitro* AST of the isolates are described in Tables

5. Isolates resistant to sulfamethoxazole/trimethoprim 20% (4/20) and gentamicin 15 (3/20) were observed, along with partially resistant strains 25% (5/20) and strains resistant 10% (2/20) to ceftiofur.

Treatment

The treatment generally adopted for these cases consisted of fluid therapy (lactated Ringer's solution) for replacement (according to the degree of dehydration) and maintenance (50 - 150 mL/kg/h) for all horses. Acid-base imbalances were corrected with electrolyte replacement as needed. Concerning the antimicrobial drugs used, the combination of potassium penicillin (40,000 IU/kg/IV/QID) and amikacin (25 mg/kg/IV/SID) was used in 36% (8/22) of the horses, and ceftiofur sodium (10 mg/kg/IV/SID) and amikacin (20 mg/kg/IV/SID) were used in 23% (5/22) of the horses. Ceftiofur sodium alone (10 mg/kg/IV/SID) was used in 14% (3/22) of the patients. In the remaining cases 27% (6/22), other antimicrobial combinations were used (e.g., gentamicin, rifampicin, azithromycin, metronidazole). Pain control was performed with flunixin meglumine (1.1 mg/kg/IV/SID) in 41% (9/22) of the cases, meloxicam (0.5 mg/kg/IV/SID) in 36% (8/22), sodium dipyrone (25 mg/kg/BID) in 14% (3/22), and dexamethasone (0.05 mg/kg/IV/SID) in 9% (2/22).

Outcome and post mortem *evaluation*

Regarding the length of hospitalization until the outcome, 46% (10/22) of the horses were hospitalized for up to 7 days, 32% (7/22) for 7-15 days, 13% (3/22) for 15-30 days, and 9% (2/22) for 30-60 days. In terms of the clinical outcome, 59% (13/22) of the horses either died or were euthanized, and 41% (9/22) were discharged (horses: 1, 3, 5, 6, 7, 13, 16, 16 and 16 and 17). Among foals less than 3 months old, 63% (5/8) were discharged, and 37% (3/8) died. Among the animals older than 3 months, 69% (9/13) died and 31% (4/13) were discharged.

The main findings in horses subjected to necropsy (n=13) included fibrin hemorrhagic enterocolitis, focal coagulative necrosis in the right dorsal colon, and segmental enteritis. Histologically, diffuse mucosal necrosis associated with a mixed inflammatory infiltrate (neutrophils, lymphocytes, and plasma cells) in the mucosa and submucosa of the colon was detected, along with diffuse congestion (Figure 1).

Table 1. Hematological values of horses under 3 months of age (n= 8) admitted with diarrhea and diagnosed with salmonellosis at a veterinary teaching hospital (FMVZ-UNESP), Botucatu, São Paulo, Brazil (2009 to 2019).

Horse	Age	PCV (%)	Red blood cells (10^6/µL)	Hemoglobin (g/dL)	PP (g/dL)	Fibrinogen (mg/dL)	Leucocytes (10^3/µL)	Band neutrophil (10^3/µL)	Neutrophil (10^3/µL)	Lymphocyte (10^3/µL)	Monocyte (10^3/µL)	NLR
2 - Death	14 days	46	9.94	14.7	6.8	800	3.6	0	1.5	1.8	0.3	0,83
3 - Discharged	24 days	42	8.52	14.7	8.6	800	7.7	0	5.7	1.8	0.2	3,17
4- Discharged	1 month	25	4.82	8.2	7.0	1000	18.5	0	16.1	2.2	0.2	7,32
5- Discharged	1 month	28	6.6	9.3	8.0	600	5.6	0.3	2.3	1.8	1.2	1,28
6 – Discharged	1 month	54	NP	20.5	7.2	400	4.4	0	3.5	0.7	0.2	5,00
7 - Discharged	2 months	29	7.4	9.2	6.4	400	13	0	11.7	1.2	0.1	9,75
8 – Death	2 months	33	9.46	11.4	6.6	600	7.6	0	4.6	2.2	0.8	2,09
9- Death	3 months	28	8.9	9.8	5.6	600	14.4	0	10.5	3.0	0.9	3,50
Mean±std dev	General	35.6±9.8	7.9±1.7	12.2±3.9	7.0±0.9	551±276	9.4±5.0	0.1±0.1	7.0±4.9	1.8±0.6	0.5±0.4	4,1±2,9
Median		31	8.5	10.6	6.9	600	7.7	0	5.2	1.8	0.3	3.3
Hematologic Values of the Foals Separated According to the Outcome												
Mean±std dev	Discharged	35.6±12.2	6.8±1.6	14±5.2[a]	7.4±0.9	481.7±360	9.8±5.9	0.1±0.4	7.9±5.9	1.5±0.6	0.4±0.5	5.3±3.3
Median		29.0	7.0	9.3	7.2	600	7.7	0	5.7	1.8	0.2	5.0
Mean±std dev	Death	35,7±9.3	9.4±0.5	12.±2.5[b]	6.3±0.6	667±115	8.5±5.5	0±0	5.5±4.6	2,3±0.6	0,7±0.3	2.1±1.3
Median		33.0	9.5	11.4	6.6	600	7.6	0	4.6	2.2	0.3	2.1
Reference value[a]		34.2±42.4	10.6-13.2	11.8-15.0	6.0-6.8	410-520	7.83-13.77	0.0-0.1	3.9-8.7	3.68-6.48	0.0-0.5	

PCV: Packed Cell Volume PP: Plasma protein. NLR: neutrophil-lymphocyte ratio. a [20].

Table 2. Hematological values of horses older than 3 months of age (n= 13)) admitted with diarrhea and diagnosed with salmonellosis at a veterinary teaching hospital (FMVZ-UNESP), Botucatu, São Paulo, Brazil (2009 to 2019).

Horse	Age	PCV (%)	Red blood cells (10^6/µL)	Hemoglobin (g/dL)	PP (g/dL)	Fibrinogen (mg/dL)	Leucocytes (10^3/µL)	Band neutrophil (10^3/µL)	Neutrophil (10^3/µL)	Lymphocyte (10^3/µL)	Monocyte (10^3/µL)
10-Death	7 months	32	8.8	10.4	7.4	800	4.6	0	1.2	2.0	1.4
11-Death	9 months	36	NP	12.8	9.2	800	3.5	0	1.4	1.9	0.2
12-Death	9 months	28	NP	10.2	6.0	1000	6.4	0	3	2.9	0.5
13-Discharged	9 months	33	9.06	10.8	8.0	1000	8.7	0	5.7	1.8	0.5
14-Death	1 year	39	9.97	12.7	4.4	800	17.3	0	12.6	4.2	0.5
15-Discharged	1 year	33	7.5	10.9	6.4	800	13	0	12.4	0.5	0.1
16-Discharged	2 years	30	7.08	10.0	7.2	200	9.7	0	5.1	4.4	0
17-Discharged	4 years	32	6.16	11.5	5.6	600	30.6	0	27.2	1.2	2.1
18-Death	7 years	32	NP	11.2	5.2	600	5.8	0	4.2	0.9	0.6
19-Death	9 years	31	8.74	10.9	5.6	600	16.3	0	13.5	2.3	0.5
20-Death	10 years	35	8.81	12.2	4.2	800	21.67	0	16.5	4.1	1.1
21-Death	14 years	26	6.93	8.4	8.0	800	8.5	0	7.8	0.6	0.1
22-Death	24 years	40	8.24	13.8	5.4	400	4.9	0	3.3	1.3	0.3
Mean±std dev		32.8 ±3.8	8.1±1.1	11.2±1.4	6.4±1.4	707±216	11.6±7.02	0.0±0.0	8.8±7.2	2.2±1.4	0.6±0.6
median		32	8,49	10,9	6	800	8,7	5,7	1,9	0,5	0
Hematologic Values of the Foals Separated According to the Outcome											
Mean±std dev	Discharged	32±1.4	7.45±1.2	10.8±0.6	6.8±1.03	650±341	15.5±10.2	0.0±0.0	12.6±10.8	1.98±1.7	0.7±0.7
		32.5	7.3	10.85	6.8	700	11.35	12.6	9.05	1.5	0.3
Mean±std dev	Death	33±4.7	8.58±4.45	11.4±1.7	6.15±1.7	733±173	9.8±6.7	0.0±0.0	7.0±6.7	2.2±1.3	0.6±0.4
Median		32	8.8	11.2	5.6	800	6.4	6.4	4.2	2,0	0.5
Reference value[a,b,c]		34-49	6.0-13.0	10.6–-18.9	5.8-8.7	100-400	5.2–-13.8	0.0-0.1	2.27-8.5	1.1-6.8	0.0-1.4

PCV: Packed Cell Volume PP: Plasma protein. a [20] b [15] c [13].

Table 3. Serum biochemistry values of horses of all ages (n=15) admitted with diarrhea and diagnosed with salmonellosis at a veterinary teaching (FMVZ-UNESP), Botucatu, São Paulo, Brazil (2009 to 2019).

Horse	Age	Urea (mg/dL)	Creatinine (mg/dL)	Aspartate aminotransferase (AST) (UI/L)	Gamma-glutamyl transferase (GGT) (UI/L)	Alkaline phosphatase (ALP) (UI/L)	Total Serum protein (TSP) (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Total bilirubin (UI/L)	Conjugated bilirubin (UI/L)	Unconjugated bilirubin (UI/L)
2-Death	1 month	68.2	0.8	NP	NP	NP	NP	NP	NP	NP	NP	NP
4-Death	1 month	429	14.96	161	23.0	128	5.5	2.2	3.3	NP	NP	NP
5-Discharged	1 month	47	0.7	90	14.6	295	6.9	2.3	4.6	3.32	0.82	2.5
6-Discharged	1 month	303.4	6.71	100	4.0	170	5.4	2.4	3	4.18	1.47	2.71
7-Discharged	2 months	67	1.2	387	199	199	8.1	2.6	5.5	2.24	0.41	1.83
8-Death	2 months	20	1.26	264.9	23.2	376.8	NP	2.9	NP	2.91	0.57	2.34
9-Death	3 months	263	3.17	137	7.6	135	5.1	2.1	3	NP	NP	NP
11-Death	9 months	133	0.95	140.8	16.5	375	NP	2.6	NP	1.12	0.67	0.45
12-Death	9 months	74	1.52	229.3	23	217	NP	2.4	NP	1.76	0.68	1.08
13-Discharged	9 months	55	1.15	227.8	19.8	221.6	NP	2.6	NP	2.13	0.53	1.6
16-Discharged	2 years	65	1.54	297	10.8	NP	7.1	3.3	3.8	NP	NP	NP
17-Discharged	24 years	81	1.2	931	1304	244.7	NP	NP	NP	5.58	0.65	4.93
18-Death	7 years	42	1.28	295	9.5	298	4.5	2.1	2.4	3.7	0.2	3.5
19-Death	9 years	20	0.7	649.2	35	247.5	NP	2.7	NP	2.36	0.73	1.63
22-Death	14 years	53	2.1	208.1	17.1	235	6.2	3	3.2	3.69	0.38	3.31
Mean±std dev		114.7±120	2.61±2.7	294.1±232.4	121.9±343.7	241.7±78.7	6.1±1.1	2.55±0.4	3.6±1.0	2.9±1.2	0.64±0.32	2.35±1.25
Median		67	1.26	228.55	18.45	235	5.85	2.60	3.25	2.91	0.65	2.34
Reference value[a]		21-51	1.2-1.9	226-366	4.3-13.4	143-395	5.2-6.9	2.6-3.7	2.62–4.04	0-2	0.0-0.4	0.2-2

NP: not performed. a[27].

Table 4. Blood gas values of horses of all ages (n=6) admitted with diarrhea and diagnosed with salmonellosis at a veterinary teaching (FMVZ-UNESP), Botucatu, São Paulo, Brazil (2009 to 2019).

Horses	Age	pH	pCO_2 (mmHg)	pO_2 (mmHg)	HCO_3 (mmol/L)	BE (mmol/l)	Ânion gap (mmol/L)	Cl^- (mmol/L)	K^+ (mmol/L)	Na^+ (mmol/L)	SID_3 (mmol/L)
4-Death	1 month	7.21	40	52	14.9	-11.3	21.3	86	3.94	122	39.94
5-Discharged	1 month	7.30	34.3	58.1	19.8	-5.4	12.1	127	2.26	157	32.26
6-Discharged	1 month	7.20	40	38.4	17.6	-9.2	13	98	2.15	129	33.15
9-Death	3 months	6.90	33.6	37	6.7	-23.3	26.9	101	3.25	135	37.25
16-Discharged	2 years	7.30	46.8	42.1	27.1	2.3	19.3	108	3.71	151	46.71
17-Discharged	4 years	7.40	30	47	22.3	-1.1	15	104	2.5	139	37.5
Mean±std dev		7.218±0.172	37.45±6.0	45.3±7.63	21.7±4.08	-8±9.0	17.9±5.2	104±12.4	2.97±0.77	138.3±12.5	37.8±5.3
Median		7.255	37.15	44.55	18.7	-7,3	17.5	102.5	2.88	137	37.38
Reference value[a,b]		7.3-7.4	38-46	38-42	24-30	±5	8-13	99-109	2.6-5.0	132-146	±40[c]

a [27]b [23] c[17]

Table 5. Distribution of the antimicrobial sensitivity profile of *Salmonella* spp. isolates in horses (n=22) attended at teaching veterinary hospital (FMVZ-UNESP), Botucatu, São Paulo, Brazil (2009 to 2019).

Classes	Antimicrobial	Number of tested isolates	Susceptible (%)	Intermediate (%)	Resistant (%)
Aminoglycosides	Amikacin (AMK)	14	12 (85)	2 (15)	0 (0)
	Gentamicin (GEN)	20	16 (80)	1 (5)	3 (15)
Beta-lactamic and derivates	Amoxicillin (AMX)	7	6 (85)	0 (0)	1 (15)
	Ampicillin (AMP)	13	12 (92)	0 (0)	1 (8)
	Cefalexin (LEX)	4	4 (100)	0 (0)	0 (0)
	Ceftiofur (CFUR)	20	13 (65)	5 (25)	2 (10)
	Ceftriaxone (CTR)	6	4 (66)	1 (22)	1 (22)
Macrolides	Azithromycin (AZM)	1	0 (0)	0 (0)	1 (100)
Quinolones /Fluoroquinolones	Ciprofloxacin (CIP)	13	13 (100)	0 (0)	0 (0)
	Enrofloxacin (ENO)	18	13 (72)	4 (22)	1 (6)
	Levofloxacin (LVX)	2	2 (100)	0 (0)	0 (0)
	Marbofloxacina (MBF)	1	1 (100)	0 (0)	0 (0)
	Norfloxacin (NOR)	13	13 (100)	0 (0)	0 (0)
Phenicols	Chloramphenicol (CHL)	2	2 (100)	0 (0)	0 (0)
	Florfenicol (FFC)	17	17 (100)	0 (0)	0 (0)
Sulfonamides	Sulfamethoxazole/Trimethoprim (SXT)	20	16 (80)	0 (0)	4 (20)
Tetracyclines	Tetracycline (TET)	7	6 (85)	0 (0)	1 (15)

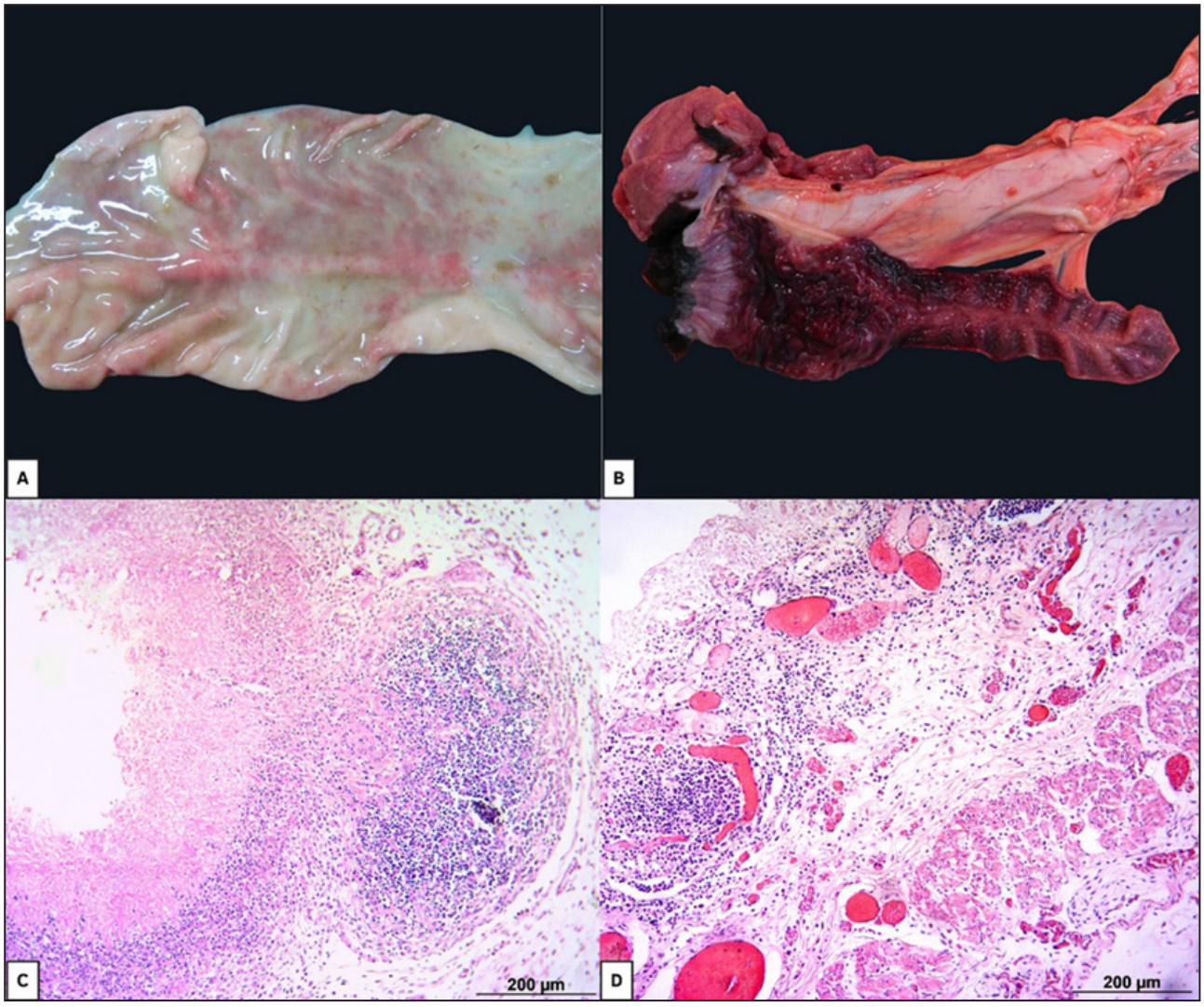

Figure 1. Intestinal anatomopathological changes in equine salmonellosis. A- Gross view of enteritis with mucosal edema. B- Gross necro hemorrhagic and hyperemic aspect of rectal mucosa. C- Small intestine. Severe and diffuse villus necrosis marked inflammation (lymphoplasmacytic) in lamina propria and edema in submucosa [HE; obj.100x]. D- Large intestine. There is diffuse mucosal necrosis, marked lamina propria and submucosal edema, inflammation, and congestion [HE; obj.100x].

DISCUSSION

Among the horses admitted during this period, gastrointestinal disorders accounted for 10% (78 out of 823) of the cases treated by the hospital. Among these cases, salmonellosis represented 28% (22/78) of the cases, suggesting that salmonellosis is a frequent infectious cause of diarrhea in horses [40,50,56]. The greater number of cases in young animals (up to 12 months) 68% (15/22) is similar to what has been described in the literature [31,50] and highlights the importance of this pathogen in young horses.

The main clinical signs (diarrhea, fever, and lethargy) observed in this retrospective study have been commonly described in horses with salmonellosis [6]. They are caused mainly by systemic abnormalities resulting from dehydration, metabolic acidosis, systemic and local inflammation, and the translocation of the agent from the gut to the bloodstream [6,25,56].

Hematological analysis revealed neutrophilia in 43% (9/21) of the horses. This abnormality is associated with the development of inflammation in the intestinal mucosa (enteritis, typhlitis, and colitis) and increased intestinal permeability, leading to macro-

phage invasion and systemic dissemination of bacteria [28,46,56]. On the other hand, neutropenia was observed in 2 horses older than 3 months of age 15% (2/8) and 3 horses younger than 3 months of age 37% (5/13), and neutrophilia in 38% (3/8) and 46% (6/13) of the 2 age groups was more frequently observed in severe and acute cases of salmonellosis, respectively [52]. An increase in fibrinogen was observed in 81% (17/21) of the horses, 75% (6/8) of the foals under 3 months, and 85% (11/13) of the horses over 3 months, confirming the presence of ongoing inflammation in these animals [52].

The NLR values observed in the foals were similar to those described in hospitalized foals [45]. When divided into 2 groups, the foals that were discharged had NLR values close to those described in healthy animals, whereas the animals that died had values similar to those of septic foals [19,45]. All foals were classified as non-septic at the time of admission on the basis of the sepsis score applied [19].

The serum urea concentration marginally increased in most animals, which could be explained by dehydration and the development of the early stages of sepsis and acute kidney injury. The creatinine values remained within the normal range in most animals (80%), except in three horses that presented with azotemia. These findings indicate that renal evaluation should be considered for horses with salmonellosis, along with additional examinations such as urinalysis [27,37].

The serum biochemistry findings in this study were similar to those reported in a previous study [52]. Liver enzyme activity is commonly increased in severe cases of salmonellosis, resulting from hepatic damage due to the filtration of bacterial toxins from the portal circulation [52]. However, the increase in AST activity can also be attributed to muscle injury [27].

Blood gas analysis revealed metabolic acidosis due to the accumulation of organic acids and electrolyte disturbances. These results arise from the significant loss of fluids and electrolytes in the feces. Although not measured, the horses likely had hyperlactatemia, as indicated by the elevated anion gap (17.9±5.2 mmol/L). This response is attributed to hypovolemia and consequently anaerobic metabolism, resulting in lactic acid production in tissues [7,16-18,52]. Despite the electrolyte concentrations being within the reference range, an alteration in the sodium ratio was observed, as evidenced by the reduction in the SID3 value [7,16-18].

The short hospitalization period observed in animals with septic shock due to death or euthanasia limits the ability to investigate the relationship between hospitalization length and prognosis [1]. This study's high mortality rate 59% (13/22) is consistent with similar studies in horses with bacterial enterocolitis [16-18,30]. These effects result from the worsening of systemic alterations and compromised vital functions [5,6,37].

In the present study, the etiology of diarrhea was confirmed by isolating *Salmonella* spp. Serial fecal culture, with three to five samples collected at 12-24 h intervals, is considered the gold standard for the diagnosis of equine salmonellosis [12,51]. However, isolating the agent in only one sample from horses with gastrointestinal alterations is sufficient for determining the diagnosis [5,6]. In addition, isolation of the agent from tissue samples and blood indicates systemic dissemination of the agent [58].

The resistance profile observed in this study, without the isolation of multidrug-resistant strains, differs from what has been described in studies with foals [24], adult horses [10], calves [36] and pigs [48]. These results may be associated with antimicrobial use practices in different countries and the regulation of their sale.

The antimicrobial drugs to which the isolates presented the highest proportion of resistance were sulfamethoxazole/trimethoprim (SXT), gentamicin (GEN), and ceftiofur (CFT). These results are similar to those described in hospitalized horses [10,32].

The observation of partially sensitive isolates (25%) or those resistant (10%) to ceftiofur should be highlighted. Other studies have described the presence of *Salmonella* spp. strains resistant to cephalosporins and aminoglycosides in foals [2,3,11]. The widespread use of cephalosporins in veterinary medicine may be associated with bacterial resistance in the future [2,3,32,59].

The findings observed via necropsy are similar to those described in cases of equine salmonellosis [56] and in horses with bacterial enterocolitis [60] This leads to the loss of intestinal permeability, favoring bacterial dissemination and the onset of sepsis [24].

The limitations of this study include the lack of serotyping identification of isolates or molecular characterization, the absence of epidemiological data (previous history of medication use, sanitary changes

and the involvement of other animals), the lack of tests for the detection and isolation of other etiological agents, and the small sample size. Nevertheless, this is the first study to describe the clinical, laboratory and pathological changes in equine salmonellosis, as well as the AST profile, in this geographical area.

CONCLUSION

Salmonellosis is a common cause of diarrhea in horses and is associated with high mortality in hospitalized cases. Clinical findings include diarrhea, dehydration, hyperfibrinogenemia, neutrophilia, and metabolic acidosis. Antimicrobial resistance was highest for sulfamethoxazole/trimethoprim, gentamicin, and ceftiofur, emphasizing the importance of rational antimicrobial use in treatment strategies.

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