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Endotoxemia in Horses in Sri Lanka - Case Report

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Six thoroughbred horses (2 male and 4 female) aged 6 to 12 years from an upcountry stable were brought between June, 2011 to Jan 2012, to the Veterinary Teaching Hospital (VTH) with signs of mild to moderate abdominal colic. On arrival, the animals were pyrexic (36.61 ˚C to 39 ˚C), tachycardiac (72/min to 100/min), tachypnoeic (15/min to 60/min) and the mucous membranes were muddy colored. The capillary refilling time was more than 2 seconds. The treatment protocol consisted of vigorous fluid therapy (0.9% Sodium Chloride Solution and 5% glucose solution), gastric decompression using a stomach tube, and administration of Flunixinmeglumine (1.1mg/kg, IV), Penicillin and Streptomycin (20/20LA, 20ml, IM) and in 03 cases ceftriaxone® (50 mg/kg IV, bid). The hematology in all cases revealed haemo-concentration with an increased packed cell volume and elevated serum protein. There was also a neutrophilic leukocytosis with a left shift. Despite treatments, 4 of 6 animals succumbed soon after arrival and necropsies revealed a frothy secretion in the trachea and bronchi, pulmonary emphysema and diffuse ecchymotic hemorrhages in lungs. There were subepicardial and subendocardial petechiation. The stomach contents were red and watery. There was hepato-splenic congestion, hyperaemia of the small intestines and diffused red, discoloration of the mucosa of the caecum and ascending colon. Histopathology of the intestines revealed diapedesis and denuded epithelial surfaces. Hepatic venous congestion with leukocytes infiltration confirmed moderate hepatitis. Pure colonies of non-hemolytic strains of E. coli were isolated from the heart blood in two cases. These clinical and post mortem findings were consistent with acute severe typhylocolitis with consequent endotoxaemia. A diet high in concentrates and low in fiber was thought to have precipitated the condition. As a preventative measure, a dietary change with ad libitum good quality hay/grass with reduced concentrates was recommended.

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INTRODUCTION

Typhlocolitis, the inflammation of caecum and colon, is a common and often fatal disease process in horses. Diarrhea consequent to altered secretory and absorptive capacity of the inflamed colon and caecum is the most common clinical sign associated with typhilocolitis. There are many causes of typhlocolities but the common aetiologies include bacterial infection (Salmonella and Clostridium species), prolonged antimicrobial therapy, non-steroidal anti-inflammatory drug (NSAID) toxicity, and intestinal parasitism [3]. Further sudden changes in feed or the ingestion of improperly balanced daily ration of feed can alter microbial flora of large intestine leading to bacterial overgrowth especially by enterotoxigenic Clostridium species resulting in typhilocolitis [12][13][14]. Profound inflammation of the large intestine s, especially endotoxins from the out cell wall of Gram negative bacteria which results in pro inflammatory cytokine (IL-1,IL-6.TNF-alpha) mediated systemic changes such as, leucopenia followed by leukocytosis ,hypovolemia, increased endothelial permeability and acid base imbalance. In the acute and severe form of typhlocolitis death can occurs even before

diarrhea is evident. In chronic form of the disease protein loosing enteropathy will often result in gastrointestinal edema.

Typhlocolitis is reported worldwide in all breeds horses [9, 5, 12, 13, 8, 14] Diarrhea has been recognized as an apparent clinical sign at the onset of the disease. This clinical communication describes the history, clinical signs, necropsy findings, diagnosis and treatment of acute typhylocolits in a group of thoroughbred horses from a single stable.

CASE STUDY

Six thoroughbred horses (6-12 yrs, 2 stallions and 4 mares) from stable in the upcountry region above sea level (1200m – 2500m) with signs of mild to moderate colic were presented to the Veterinary Teaching Hospital (VTH) of the University of Peradeniya between June 2011 to January 2012.

All the animals presented to VTH had developed mild to moderate colic 12-24 hours earlier before. On arrival a general clinical examination was performed. Jugular blood was also collected for hematology. Nasogastric tube was passed to decompress the stomach. Initial examination revealed that the animals had elevated temperature (39 ˚C), heart (72-100 bpm) and respiratory (15- 60 breaths/min) rates. The mucous membranes were muddy brown coloured and the capillary refilling time was in excess of 2 seconds. No defecation was observed during the course of the disease and scant feces were found in the rectum of 3 horses. The treatment protocol of the affected horses consisted of initial of aggressive fluid administration [0.9% Sodium Chloride Solution, 5% glucose solution (0.5ml/sec)] and Flunixinmeglumine (1.1mg/kg, IV). This was followed by IM injection of Penicillin and Streptomycin combination (20/20LA, 20ml) in first 2 cases and in latter 03 cases, ceftriaxone (50 mg/kg IV, bid).

On admission Case 1 was administered with IV fluid, buscopancompositum (20ml, 4mg/ml, IV) and Pen-Strep.Case 2 was given flunixinmeglumine (2.2mg/Kg, IM) in addition to treatment described for Case 1. Case 3 had been administered with 2 liters of 0.9% NaCl intravenously at the stable. Case 4 was given flunixinmeglumine IM, Ceftriaxone® (50mg/Kg) IV along with the IV fluid. The first three cases presented at the onset of the episode did not recover. The next two cases (4 and 5) recovered after treatment and were discharged at a later date. Case 6 died 12 hours after admission

Detailed postmortem examinations were conducted soon after death of animals. Tissue samples of the kidney, spleen, liver and lungs were collected in 10 percent neutral buffered formalin for histo-pathological examination. Part of the gut was collected from (Case 2) for isolation of bacteria and endotoxins. Heart blood swabs were also collected for bacterial culture.

RESULTS

Clinical findings in the six horses at the time of presentation are summarized in table 1.

Hematology findings of all the cases are summarized in Table 2. The most striking feature of hematology was severe dehydration as indicated by increased PCV and plasma proteins. Leukocytosis with left shift was observed in 3 horses. The faecal parasite eggs counts were negative in all animals. Gastric reflex was not observed in any of the cases.

The necropsy findings of all four horses showed a frothy white secretion in the trachea and the large and small bronchi and bronchioles with in the lung parenchyma. There was also marked locally extensive petechiation and ecchymosis of the lungs (figure 1). In the heart there was marked locally extensive sub endocardial hemorrhage with petechiation and ecchymosis at the base of the heart (figure 2). The contents of the stomach were red and watery. There was moderate hepatic and splenic congestion, hyperemia of the small intestines (figure 1) and diffuse red, discoloration of the mucosa of the caecum and ascending colon. The visceral organs appeared congested. The contents of colon were watery red. There was excessive accumulation of serosanguineous fluid in the peritoneal cavity. The horse in Case 3 had a 140 days old foetus.

Histopathology of the intestine revealed the denudation of the epithelium and marked neutrophil infiltration in the lamina propria. Histopathology of liver showed hepatic

congestion and neutrophilic infiltration confirming moderate hepatitis. Hemorrhages were also present in the cortical-medullary junction of both kidneys. Pure colonies of non-hemolytic strains of E. coli were isolated from the heart blood in two cases. The clinical, laboratory, gross and histo-pathological findings are consistent with acute severe typhylocolitis with consequent endotoxaemia.

DISCUSSION

Most of the mild colic cases responded well to a single dose of anti-spasmolytic drug suggesting some involvement of the digestive tract. Mild to moderate colic that is not responsive to conservative treatment is often caused by enteroliths and these equines are usually over 6 years of age. Large single or small multiple enteroliths have been the cause for this. Mild to severe acute inflammation of hind gut (typhylocolitis) has not been reported as a cause for colic in horses or ponies in Sri Lanka. However the present clinical signs and findings suggested that these animals have considerable vascular compromise that was not a regular finding of colic associated with eneteroliths. Necropsy findings of the first three cases were helpful to arrive at tentative diagnosis for the subsequent cases, and these were therefore treated for possible endotoxaemia caused by acute severe typhphlocolitis. The typhylocolitis in the horse can arise from a number of causes and the etiology of individual cases frequently remains obscured despite vigorous diagnostic attempts[11].

Diagnosis of endotoxemia secondary to typhlocolitis was based on the clinical signs such as mild to moderate colic, depressed appearance, elevated rectal temperature, tacky mucous membranes, decreased gastrointestinal movements, tachycardia, pulse of small amplitude, muscle weakness, and increased capillary refilling time[3]. Diarrhea developed due to microbial population was a common finding 1n previously reported typhlocolitis in thoroughbred horses [14, 13, 9]. None of the affected horses in present study developed diarrhea as a major clinical sign. Leukocytosis with neutrophilia, high serum total protein with increased globulin levels is also suggestive of

endotoxemia [11]. Gross and histopathological findings were useful in confirming endotoxemia secondary to typhylocolitis in dead animals. The gross necropsy findings were due to lesions caused by toxins and dehydration. Histopathological findings also reflect the tissue response to toxins..

Accumulation of fluid in the intestine is due to increased secretory activity and decreased absorptive capacity of colon. This might have contributed to reduce intestinal motility. Circulatory failure was a direct result of hypovolemia caused by sequestration of fluid in the gut. This is characterized by the increase CRT and brick red mucosae. Endotoxins also cause dilation of blood vessels that contribute to the fluid loss to interstitial space. Excessive fluid accumulation in the stomach caused rupture of the stomach in case 6 and this probably was the cause for immediate death in this horse.

Endotoxins cause mucosal injury and enterocyte necrosis which cause hyperemic small intestine and diffuse red discoloration of the mucosa of caecum and ascending colon. Toxins diffuse throughout systemic circulation beginning from the portal circulation. This initiate release of cytokines that cause endothelial damage and platelet activation leading to disseminated intravascular coagulation throughout the body. Fibrin deposition and inadequate fibrinolysis occur in organs. This causes microscopic and gross changes in lungs, heart, liver, spleen, kidney and adrenal glands. Later consumption coagulopathy occurs that leads to hemorrhagic diatheses in congestion of organs.

The culturing of heart blood in Cases 1 and 3 resulted in pure colonies of non-hemolytic *E. coli* strains. The PM was done immediately after death. The *E coli* strains are known to produce both endotoxins and exotoxins. *E. coli* are gram negative commensal bacteria found in the gut of the horses. There are many strains of bacteria under normal circumstances these organisms do not cause any infection [6].

Pharmacological basis for treatment of endotoxaemia is well described [9,10]. Briefly, the treatment protocol should include prevention of further absorption of toxin to the circulation, neutralization of absorbed toxins, prevention of

synthesis and release of endotoxins and finally the prevention of endotoxin induced circular activation through inflammatory mediators. The treatment of endotoxaemia therefore includes administration of intravenous fluid, steroidal or non-steroidal anti-inflammatory drugs and toxin binders. Usage of broad spectrum antimicrobials is also indicated. NSAIDS are generally used for its anti-inflammatory properties and modulation of hemodynamic properties. Flunixinmeglumine (0.25-1.1mg/kg) is the commonly used NSAID [3]. Polymixin B (1000-5000 units/kg) bid or tid is used as an antibiotic of choice for more resistant forms of gram negative bacterial infection. It also binds with endotoxins preventing interaction of endotoxins with white blood cells [1]. Fluid therapy using hypertonic saline increases the blood volume by drawing fluid from interstitial space. It is also possible to administer colloids along with saline that provide better results. Plasma substitutes such as Hetastarch can be administered along with a hypertonic solution for better results [4]. It has been shown that Polymixin given at lower dose can maintain endotoxin-binding property [2] there by reducing side effect of it. The main reason for including potent antibiotic in our treatment plan was to prevent septicemia caused by gram-negative bacteria. Ceftriaxone is a third generation antibiotic which is known to have a good anti-microbial effect against gram negative Enterobacteriaceae. Treatment was started at a higher dose (50mg/kg, bid) than the normally recommended dose due to severity of the infection. Two days after treatment all the clinical parameters became normal. In case 5, combination of penicillin and streptomycin was adequate to control the infection. The treatment protocol described in the present clinical communication seems to be adequate if affected animals are presented in time for treatment. However the treatment protocol could have been further improved if a toxin-neutralizing drug was used in combination with antibiotic therapy. Inappropriate ration formulation can also contribute to occurrence of typhlocolitis. Concentrate feeds formulated using barley and oat can be tolerated by equine species better than starch of maize or wheat [7]. Starch present in barley and oat will be broken down in the foregut gut and absorbed subsequently before it reaches the hindgut. Diets containing maize and wheat will reach the hindgut mostly undigested. This starch ferments in the hindgut altering the gut pH there by disturbing its function. These conditions would help rapid multiplication of gram-negative commensal organisms in the gut and invade the circulatory system causing toxaemia and bacteremia.

CONCLUSION
Since all these cases were reported from a single stable it can be hypothesized that the condition might have been triggered by feeding practices. As the main component of the ration is maize based the owner was asked to divide the ration and offer it at two occasions at 8-10 hours interval. Horse keepers were also instructed to seek veterinary care and assistance immediately after a horse becomes sick, as prognosis is always better if treatment is instituted at the onset of a disease.

AUTHORS CONTRIBUTION
All the authors have contributed equally.

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TABLES

Table 1: Clinical findings of 6 horses at admission to the VTH; the reference interval of each clinical parameter is given within parenthesis

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
Temperature	38˚C	38.5˚C	NR	36.6˚C	37.5˚C	39˚C
Pulse	40/min	NR	NR	100/min	100/min	72/min
Heart rate	40/min	Tachycardia	Tachycardia	100/min	100/min	72/min
Respiration	15/min	NR	Tachypnoea	60/min	60/min	60/min
Mucosae	Hyperemic	Congested	Hyperemic	Hyperemic	Hyperemic	Hyperemic
CRT (2 sec or <2 sec)	2 seconds	>2 seconds	>2 seconds	-	>2 seconds	3 seconds
Skin	-	Dull coat	Excessive sweating	-	-	Healthy coat
Faeces	-	-	-	Projectile, watery diarrhea	Dry scanty faeces	Soft scanty faeces
Other	Enlarged abdomen, Show colic signs	Severely dehydrated. Colic signs present.	Colic signs present	Enlarged abdomen Colic signs present	Dark colour Urine	Dark colour urine

NR – Not recorded   CRT- Capillary refilling time

Table 2: Hematological parameters of samples collected at the time of admission.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Normal values
PCV % (L/L)	45	70	96	57	60	72	(32-53)
RBC count	-	15.65×10^6	-	12.63×10^6		14.53×10^6	$(6.8\text{-}12.9) \times 10^6$
RBC morphology	Poikelocytes Spherocytes		-	Acanthocytes Poikelocytes		Echinocytes	
WBC count	9000/µl	13,300/µl	-	20,800/µl	14,750/µl	16,260/µl	(5400-14,300)
Neutrophils /L ($\times 10^{-6}$)	6480	-	-	18720	12537.5	15284.4	
Lymphocytes /L ($\times 10^{-6}$)	2250	-	-	832	1327.5	650.4	
Total protein (g/L)	-	0.975	-	L 0.831	0.986	0.664	(0.57-0.79)
Albumin (g/L)	-	0.379	-	0.426	0.343	0.29	(0.25-0.38)
Globulin (g/L)	-		-	0.405	0.643	0.374	(0.23-0.46)

FIGURES

Figure 1. Changes in the intestine

Figure1.1.Case 6- Hyperemic Intestinal
Mucosae

Figure 1.2 .Case 4-Rupture point of the stomach,
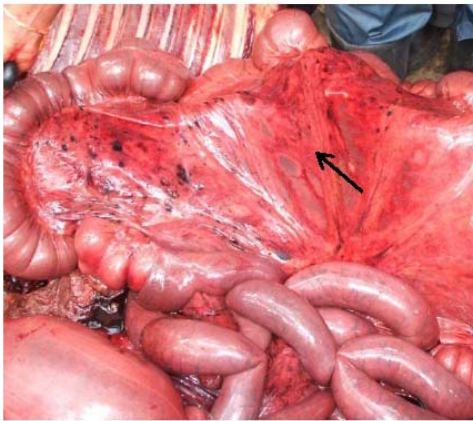hemorrhagic line around the border is present

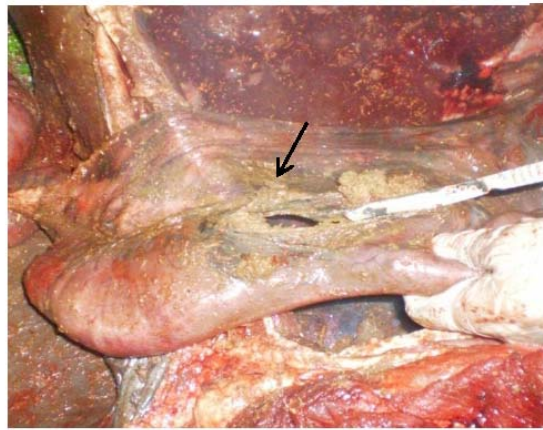Figure1.3. Case 2-Hemorrhages in the
mesentery blood vessels

Figure1.4 .Case 3-Rupture point of transverse colon
hemorrhagic line around the border is present

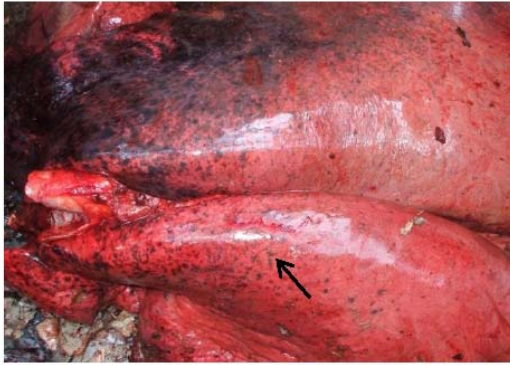**Figure 2.** Changes in other organs

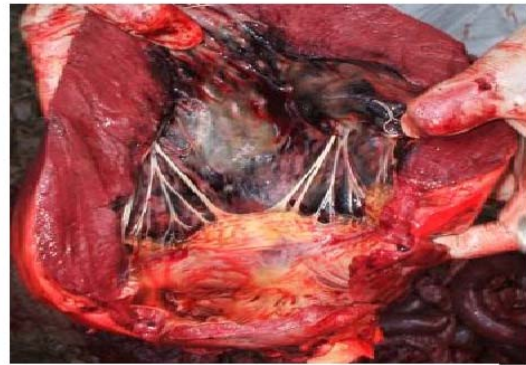Figure 2.1.Case 3-Diffuse pettichiation in lung lobes

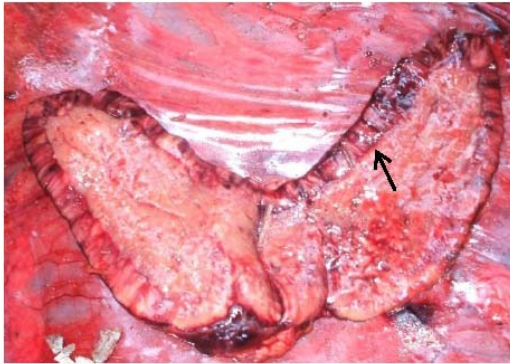Figure 2.2.Case 6-Hemorrhages in the endocardium

Figure 2.3. Case 6-Adrenal glands hemorrages indicating damage due to endotoxemia

Figure 2.4.Case 6-Heart petichiation along the coronary groove