LUPINE PUBLISHERS

ISSN: 2637-4749

Concepts of Dairy & Veterinary Sciences

DOI: 10.32474/CDVS.2020.03.000173

Research Article

Therapeutic Plan for *Clostridium Perfringens* Type A Alpha Toxin Associated Jejunal Haemorrhagic Syndrome in Bovine of Tamilnadu

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Received: June 04, 2020 **Published:** June 17, 2020

Abstract

Bovine necro hemorrhagic enteritis is an emerging economically important bovine disease caused by *Clostridium perfringens* type A. Intestinal content of three bulls affected with Jejunal hemorrhagic syndrome were subjected for toxigenic identification of Clostridium with multiplex PCR and showed positive for Clostridium Type A alpha toxin in intestine, reticulum and in aboamsum contents. These cases lead to paralytic ileus and ended in empty rectum. This research concluded that Clostridium type A alpha toxin causes necro hemorrhagic enteritis in bovines leads to empty rectum. Treatment with Sulphadimidine, charcoal and kaolin indicated good clinical recovery.

Keywords: Bovine, necro haemorrhagic enteritis, *Clostridium perfringens* type A, alpha toxin, Tamilnadu

Introduction

Bovine necro haemorrhagic enteritis is caused by *Clostridium perfringens*, which cause death with necro-haemorrhagic lesions in small intestine [1]. *Clostridium perfringens* pandemic in nature which presents in soil, sewage, food, feces and the normal intestinal microbiota of humans and animals. *C. perfringens* classified into five toxinotypes which includes A, B, C, D and E based on the presence of genes encoding four major toxins: alpha, beta, epsilon and iota toxin [2]. *C. perfringens* type A strains are the suspected etiological agent for bovine alimentary tract pathological lesions and abomasitis [3]. Even though the type A toxigenic *Clostridium perfringens* presents in the normal microflora of ruminants, risk factors like high carbohydrate, low fiber diet, low quality and quantity fiber feed will attract the disease [4]. Empty rectum caused by alpha toxigenic clostridium infection. Identification of toxin genes rather than the toxins they produce will help to identify the prevailing *C. perfringens* types in bovine cases [5]. Polymerase chain reaction (PCR) will be the better diagnostic method to demonstrate the encoding genes in these bacteria [6]. This paper focuses the epidemiological risk factors, clinical course and therapeutic management of necro hemorrhagic type A toxigenic *C. perfringens* in bovine of Tamil nadu in India.

Materials and Methods

Location

This research was carried in an organized cattle farm located in Udhagamandalam coordinates with 11.4064° N, 76.6932° E situated in southern peninsula of India. The animals were fed with green grass, roughage and concentrate in the sub recommended level. Routine deworming and vaccination were carried out.

Clinical and Laboratory Examination

A Holstein Friesian crossbred 2 year-old bull (Bull no:1) with clinical history of anorexia, dull, hemorrhagic enteritis, depressed with normal body temperature was brought for expert opinion. Routine clinical examination and rumen fluid examination were carried out. Clinical samples like whole blood, serum, dung and peripheral blood smear were collected. Medical management with parental administration of Streptopenicillin, the animal was died and detailed postmortem was carried out. Complete blood counts were analyzed with whole blood. Samples from stomach and intestinal content, vital organs were collected for toxin identification; tissues were fixed in 10% formalin for histopathology. The second animal (bull no: 2) was 2 years and 6 months old also showed hemorrhagic enteritis (Figure 1). Third animal (bull no: 3) was 3 years old; also showed hemorrhagic enteritis and dung samples were subjected for toxin identification. The intestinal contents and dung samples were inoculated into Robertson's cooked meat medium with brain heart infusion broth and incubated at 37°C for 24 h in McIntosh Field's anaerobic jar containing Anaero-Higas pack with indicator. The DNA was extracted from *C. perfringens* by high salt treatment method described [7]. Single colonies of *C. perfringens* isolates were picked up randomly with a sterile loop and DNA was extracted. Oligonucleotide primers specific to alpha, beta and epsilon toxins of *C. perfringens* were adapted from previous workers [8] the multiplex PCR was performed using a thermo cycler (Kyratech, USA) as described [9].

Figure 1: Hemorrhagic enteritis of bull no 1 with Clostridium type A Alpha toxin.

Results

Clinical examination of bull 1 and 2 showed dull, depressed, anorexia, sunken eye ball, > 8% dehydration and ruminal atony. Body temperature was within normal range and rectal examination showed scanty dung with mucous coated. Rumen fluid examination showed few medium size protozoa per field with complete absence of small and large size protozoa. Dung samples showed negative for helminthic eggs with absence of blood protozoan etiologies. Complete blood counts showed marked nutrophilia and normal hemogram. Bull no 1 and 2s were treated with Streptopenicillin, fluid therapy and B complex injection. Animals showed poor response towards the medical management and died. Postmortem examination of intestine showed ballooning (Figure 2), necrosis, dark red in color and hemorrhagic in nature (Figures 3-6). Petechial hemorrhages noticed in auriculoventricular junction of heart (Figure 7&8) and hemorrhage was noticed in abomasum (Figures 9&10). Microscopical examination of the intestine showed hemorrhages and congestion in the mucosa and submucosa. Moderate edema and mononuclear cell infiltrations were noticed in the submucosa (Figures 11-13). The multiplex polymerase chain reaction revealed positive for type A alpha toxin of Clostrodium perfringens in recticulum and jeuenum contents and negative in ileum contents. Third animal dung was showed positive for alpha toxin of *Clostridium perfringens* and treated with parental sulphadimidine, charcoal and kaolin and eventual clinical recovery was noticed.

Figure 2: The dark brown discolored and ballooning of distended intestinal segments.

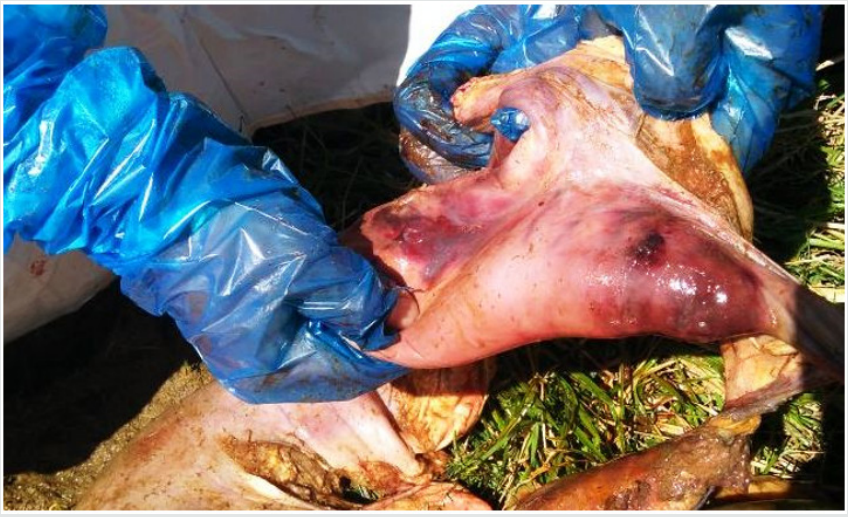

Figure 3: Dark reddish discoloration of the intestinal serosal surface indicating severe congestion (bull 1).

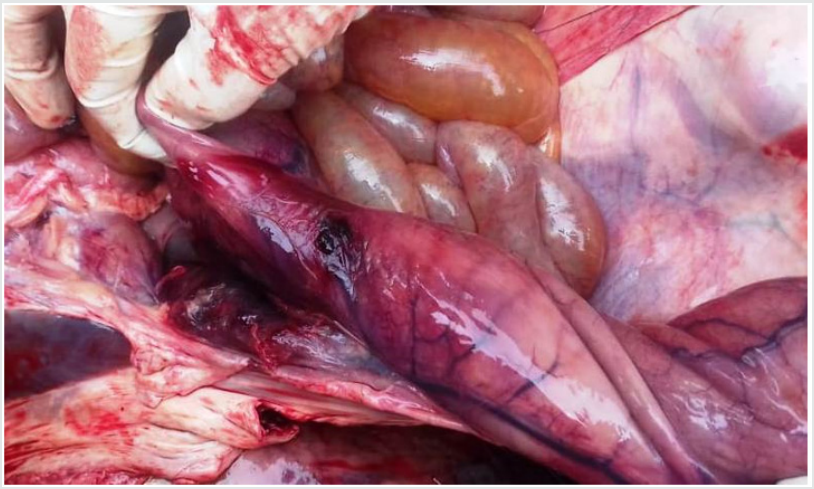

Figure 4: Dark reddish discoloration of the intestinal serosal surface indicating severe congestion and haemorrhages with engorged blood vessels (bull 2).

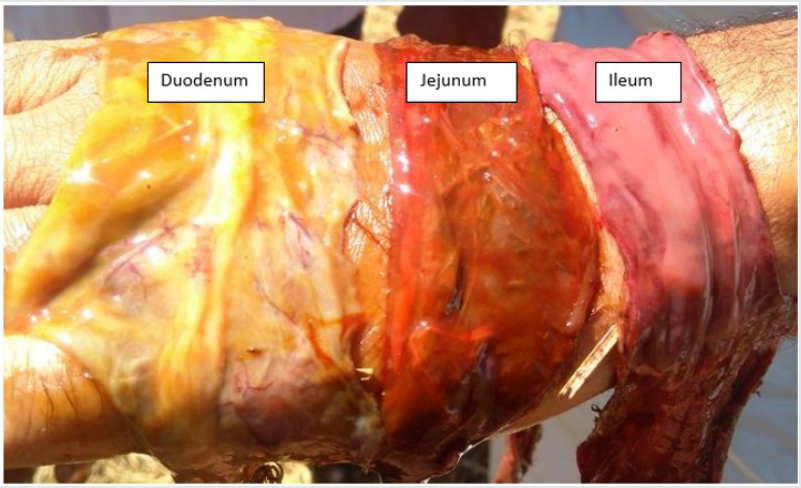

Figure 5: Diffuse severe hemorrhagic ulceration of different segments of intestine like duodenum, jejunum and ileum of bull no 1.

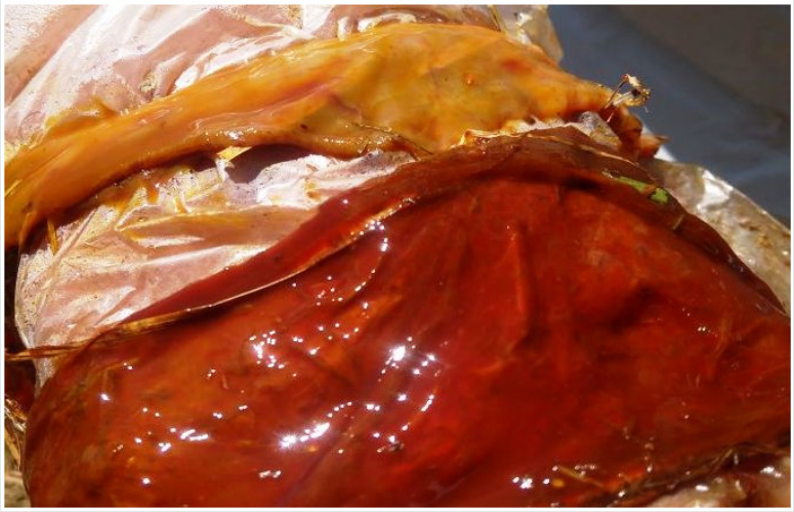

Figure 6: Severe hemorrhages with few ulcerations in the jejunum and duodenum of bull no 2.

Figure 7: Petechiael hemorrhage with engorged blood vessesls on the epicardial surface of the heart and auriculoventricular junction of bull no 1.

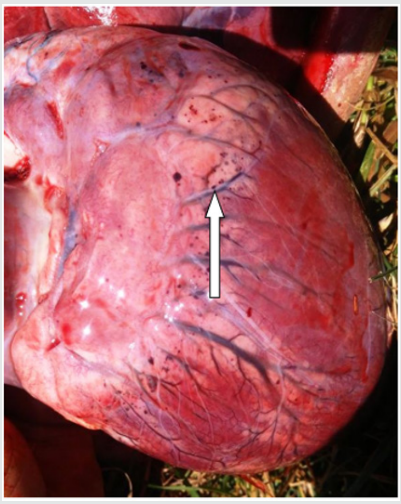

Figure 8: Petechiael hemorrhages on the auriculo ventricular junction of bull no 2.

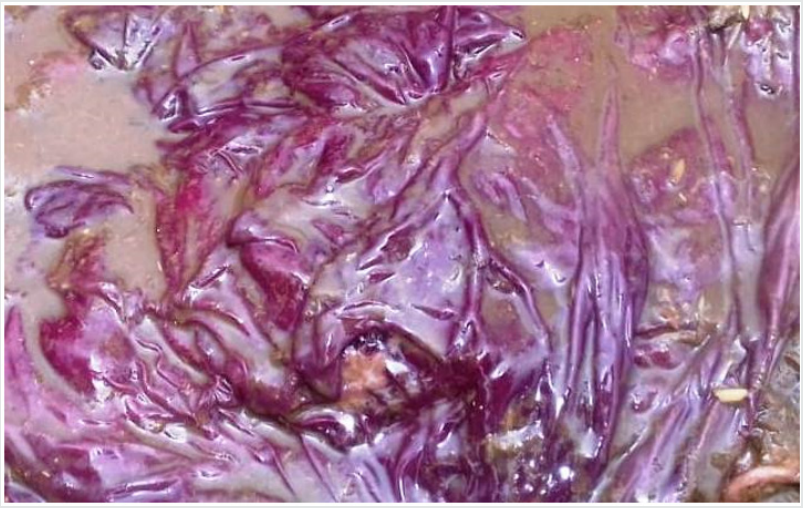

Figure 9: Purple discoloration of abomasum indicating severe congestion with ulcer and necrosis in bull no 1.

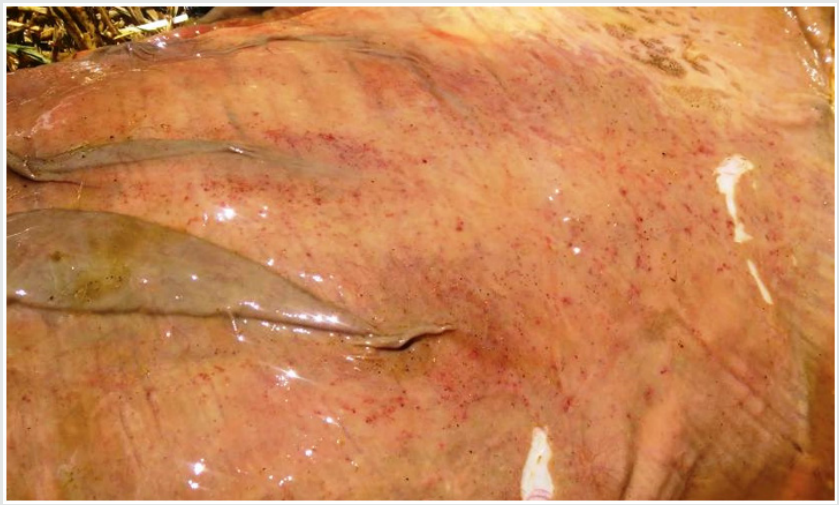

Figure 10: Petechiael haemorrhages and few linear ulcers in the abomasal mucosa of bull no 2.

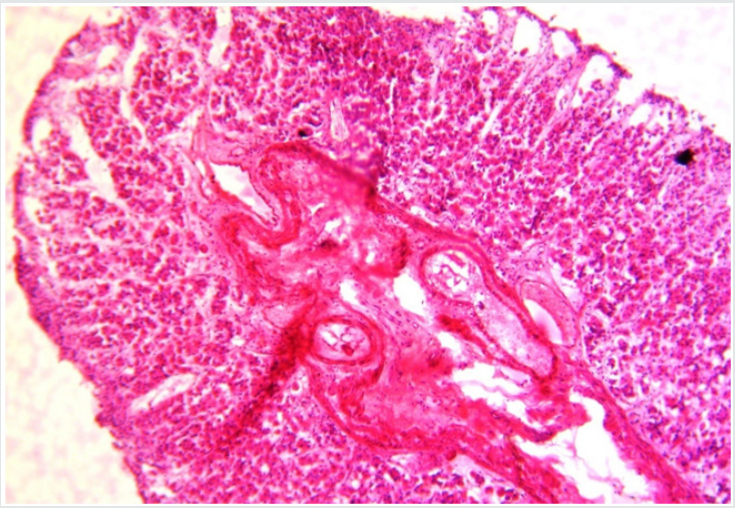

Figure 11: Histopathology of jejunum mucosa showed moderate mononuclear cell infiltration in bull no 1.

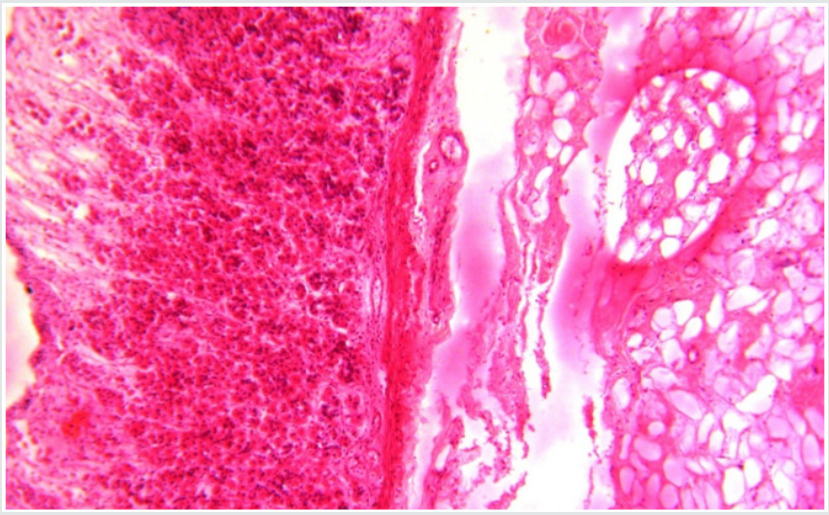

Figure 12: Histopathology of jejunum showed moderate congestion of mucosa and submucosal edema in bull no 1.

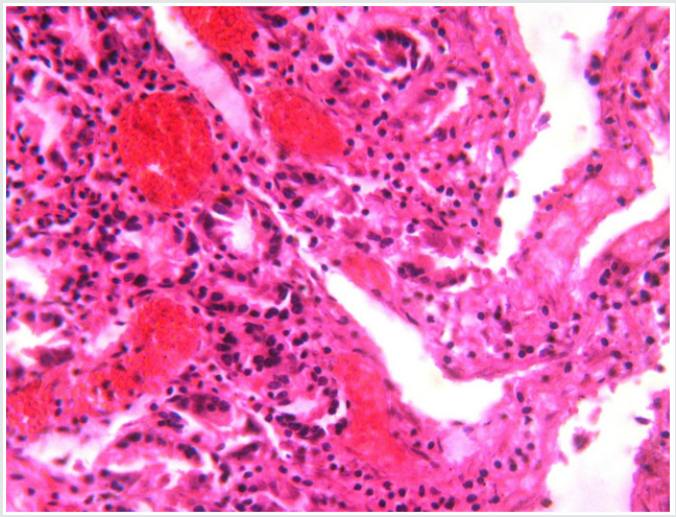

Figure 13: Histopathology of ileum showed severe congestion and mononuclear cell infiltration in the mucosa of bull no 1.

Discussion

The epidemic of the necro hemorrhagic enteritis / jejunal hemorrhagic syndrome (JHS) is a upcoming disease in cattle of southern peninsula of India. Most of the dairy farms in India following low quality and quantity fiber diet pattern. But high fiber diet would believe to protect the ruminants from gastrointestinal diseases [10]. Low quantity fiber will alter the microflora of intestinal composition to favor overgrowth of Clostridium (Table.1). Most of the cases of JHS recorded within 5 years of age [11]. As per epidemiological investigations, higher susceptibility in Holstein Friesian was due to genetic influence [11]. And recorded in good to excellent built animals [12]. First of its kind, the necro hemorrhagic enteritis by Clostridium Type An alpha toxin in India was documented. This case was eventually designated as haemorrhagic bowel syndrome (HBS) or jejunal haemorrhage syndrome (JHS) as mentioned [1and 13]. Winter season precipitate high cold condition with higher toxin production [15]. The blood profile expressed as marked nutrophila which indicated the infection in progress. The stressful risk factors like low fiber diet will cause paralytic ileus which cause abnormal changes in the microflora like expression of toxigenic genes, which produces toxin leads to intestinal hemorrhage [16]. *Clostridium perfringens* type a apha toxin was identified in recticulum, abomasum and jejunum [17]. As per the case control study model, affected animals group were purchased from northern part of India where the feed ingredients were entirely different form the present epidemic area since some of the feed ingredient would trigger the multiplication of *Clostridium perfringens* Type a alpha toxin [18]. But animals purchased from local areas where same package of practices were followed and had no/low incidence of this infection. Risk factors would increase the intestinal permeability, impair the intestinal peristalsis motility which blocks the beneficial flushing effect of intestinal transport and favors for bacterial/toxin over production [19]. Clinical sign and pathophysiology were highly complicated like intussception, volvulus and paralytic ileus depending upon the stage and severity of the pathology of Clostridium Type A alpha toxin (Table 3). On post mortem examinations of bull no 1 and 2 animals showed necrosis and hemorrhages in jejunum and abomasum (Table 1) mostly called as clostridial abomasitis [20]. The lesions of necro- haemorrhagic – jejuno - ileitis observed in cattle enterotoxaemia with toxinotype A strain was well explained by the hypothesis of a synergy between the ß1 toxin and a ß2 toxin variant [21]. Upon treatment with parental sulphadimidine @ 333 mg/kg.bwt (IV), Kaolin powder (1g/kg.bwt), activated Charcoal (0.5g/kg.bwt) orally , there was marked recovery of bill no 3. (Table 2). Risk factors included low quantity and quality fiber feed, sudden change of carbohydrate –protein source in ration would be managed efficiently to prevent the epidemic of *Clostridium perfringens* Type A alpha toxin in dairy animals.

Table 1: Chronological patho-physiology of necrotic hemorrhagic enteritis in bovines.

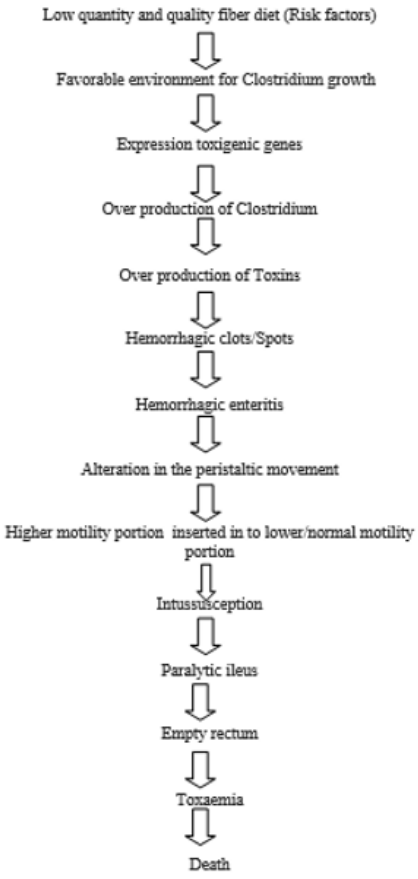

Table 2: Clinical course of necro hemorrhagic enteritis of Clostridium perfringens Type A alpha toxin in cross bred cattle.

Animal details	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 10
Bull no 1 (CBHF)	Anorexia, Normal body temperature, Ruminal atony	Anorexia, Normal body temperature, Ruminal atony	Anorexia, sub normal temperature, Ruminal atony, empty rectum	Anorexia, Sub normal temperature, Ruminal atony, downer cow, empty rectum	Anorexia, Sub normal temperature, Ruminal atony, downer cow, empty rectum	Anorexia, Sub normal temperature, Ruminal atony, downer cow, empty rectum	Died
Therapeutic plan	Inj.Streptopenicillin, intravenous fluids and supportive therapy						
Bull no 2 (HF)	Anorexia, Normal body temperature, Ruminal atony	Anorexia, Normal body temperature, Ruminal atony, empty rectum	Anorexia sub normal temperature, Ruminal atony, empty rectum	Died			
Therapeutic plan	Inj. Streptopenicillin, intravenous fluids and supportive therapy						
Bull no 3 (CBHF)	Anorexia, Normal body temperature, Ruminal atony, necro hemorrhagic enteritis (+++)	Anorexia, Normal body temperature, necro hemorrhagic enteritis (++)	Normal body temperature, Rumination present, only roughage intake, no concentrate in take	Normal body temperature, Rumination present roughage concentrate in take, complete clinical recovery	complete clinical recovery	complete clinical recovery	complete clinical recovery
Therapeutic plan	Inj.Sulphadimidine, intravenous fluids and supportive therapy includes powder kaolin and Charcoal orally						

Table 3: Comparative clinic pathology with therapeutic outcome of Jejunal hemorrhagic syndrome.

S.No	Days of Illness	Patho physiolOgy	Patho Physiology	clinical out Come	Post Mortem lesion	Percentage of success on Therapeutic Management
1	1-2	Decreased rumen motility Fail to absorb of intestinal contents	Normal rectal passage/ intestinal loops	Indigestion	NA	100%
2	2-4	Rumen motility will be nil Poor Rumen microflora Intestinal motility sluggish	Scanty dung with normal colur and consistency Intestinal loops distended	Intussusception	Intestinal hemorrhages with hemorrhagic spot/ clotMild abomasitis	75-80%
3	4-6	Complete cessation of rumen motility, no rumen microflora Intestinal motility almost nil	No dung in the rectal passage Scanty, foul smelling dung	Intussusception/ Volvulus/paralytic ileus	Severe Intestinal hemorrhages with hemorrhagic spot/clotSevere abomasitis	45-60%
4	6-8	Complete cessation of rumen motility, no rumen microflora Intestinal motility almost nil	Anorexia,Complete cessation of dung, Highly dehydrated recumbent	Intussusception/ Volvulus/paralytic ileus	Severe Intestinal hemorrhages with mucosal sloughingSevere abomasitis	30-40%

Conclusion

Clostridium perfringens Type A alpha toxigenic enteritis in cross bred cattle will have nerco hemorrhagic enteritis which is otherwise called Jejunal hemorrhagic syndrome (JHS)/ Hemorrhagic bowl syndrome. The clinical course with simple anorexia and end up in necro hemorrhagic enteritis. This case was successfully managed with parental sulphadimidine, Kaolin and activated Charcoal orally. Nowadays feeding of low quantity fiber feed and high volume of concentrated feeding practices attracts the Clostridial overgrowth which in turn produces Type A alpha toxin causes hemorrhagic inflammatory spots in intestine which pave way for intussception, obstruction, paralytic ileus and empty rectum. Upon earlier therapeutic plan with parentral Sulphadimidine, Kaolin and Charcoal orally with sufficient rehydration of fluids and electrolytes will save the animals. Avoiding risk factors included low quantity and quality fiber feed, sudden change of carbohydrate – protein source in ration will prevent the epidemic of *Clostridium perfringens* Type A alpha toxin in dairy animals.

Conflict of Interest Statement

The authors declare that they have no conflict of interest

Funding

The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing Interests

None declared.

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