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Research Article

Implication of *Clostridium perfringens* type A in Young Calves

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ABSTRACT

Clostridium perfringens produces enteric diseases in cattle, sheep and goats. The microorganism can be a normal inhabitant of the intestine of most animals and human. A total of 8 calves aged between 8 to 11 month suffering from colic, and dark clotted blood in the faeces (melena) proceeded with death. The main postmortem findings were observed as massive hemorrhage and clot formation within the small intestine as well as abomasal ulcerations. Histologically, the abomasal mucosa was sloughed and the intestinal villi appeared necrotic along with a characteristic submucosal oedema. The bacterial culture and toxin detection showed presence of *Clostridium perfringens* type A. There were no other potential microorganisms or aflatoxins have been identified.

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INTRODUCTION

Clostridium perfringens causes food poisoning and fatal enterotoxemia (Yamagishi, 1997; Yoo, 1997). Enteric *C. perfringens* infections in animals and man called enterotoxemias. There are five types of *C. perfringens* (A, B, C, D, E), which are identified by the main types of toxins they produce (alpha, beta, iota, epsilon and theta) (Niilo, 1987; Songer, 1996). *C. perfringens* type A is the most common *C. perfringens* types. It is part of the normal gut flora in cattle. However, dietary changes or parasitism may produce a favorable growth environment, resulting in overgrowth and production of potent toxins. *C. perfringens* type A can rapidly produce potent toxins, primarily alpha toxin. Alpha toxin is thought to be associated with a number of potentially deadly gastrointestinal diseases (Hatheway, 1990). Some isolates of *C. perfringens* type A produce β2–toxin which may contribute, along with α–toxin, to the development of hemorrhagic enteritis in cattle (Jelinski et al., 1995; Bueschel et al., 2003; Abutarbush and Radostits, 2005). *C. perfringens* type A is also commonly isolated in calves in cases where abomasal ulcers and abomasal haemorrhage are found (Roeder et al., 1987).

History, clinical signs, and gross postmortem findings are useful in establishing a presumptive diagnosis of clostridial enterotoxemia, but confirmation requires laboratory testing. Detection of toxins in intestine is very important to establish a diagnosis. ELISAs are considered one of the most important laboratory technique for *C. perfringens* toxins detection (Francisco and Glenn, 2008). The purpose of this paper is to present a description of *Clostridium perfringens* type A in calves that died suddenly with severe intra–luminal hemorrhage in the jejunum and abomasal ulcerations.

MATERIALS AND METHODS

Animals and Samples Collection

A total of 8 calves aged between 8 to 11 month suffering from colic, and dark clotted blood in the faeces (melena) proceeded with death was admitted to the Veterinary Teaching Hospital of the college of Veterinary Medicine and Animal resources, king Faisal, Saudi Arabia. The disease was coincided with the presentation of a new total mixed ration to animals. Blood samples were collected in heparinized tubes for determination of total and differential white blood cells count (Ve.Scan 5HM–ABaxis–USA/2002). Moreover, feedstuffs were analyzed for a total aflatoxin using a slightly modified immunoaffinity method based on Association of Official Analytic Chemists method (AOAC) (Trucksess et al., 1991).

Postmortem and Histopathology

Four cadavers were available to necropsy. Impression smears prepared by scraping intestinal mucosa and the cutting surfaces of mesenteric lymph nodes were stained by Gram's Method. Specimens of abomasums, small intestine and large intestine, and mesenteric lymph nodes were preserved in 10% neutral buffered formalin.The formalin fixed tissue samples were dehydrated through graded ethanol and embedded in paraffin blocks. Sections of 4–5 um thickness were cut and routinely stained with Haematoxylin and Eosin (HE). The selected sections were stained by Gram and Gomori methylamine silver (GMS) stains to detect bacterial or fungal organisms respectively.

Mycology and Bacteriology

The isolation of *A. fumigatus* was carried from the trachea, lungs, liver, kidney, brain; small and large intestine.These samples were directly streaked on sabouraud agar plates for culturing and were incubated for 7 days at 37 ° (Darise, 1987). *A. fumigatus* was identified according to its specific colony characteristics, slides were also prepared for identification of mycelium and hyphial arrangement with lactophenol blue staining method. Additionally, intestinal contents were cultivated in Cooked

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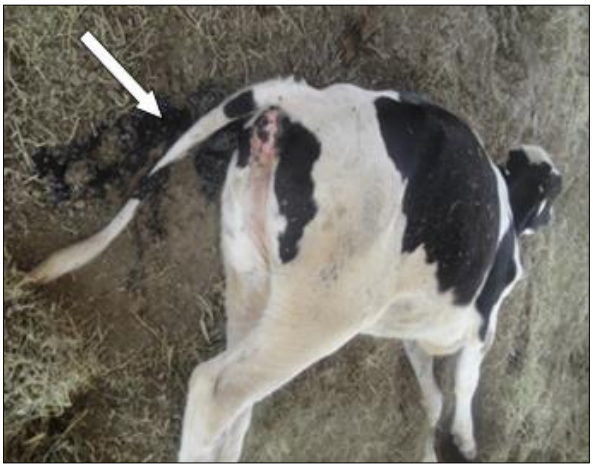
Meat Broth (CMB) and incubated anaerobically at 37°C for 48 hours. From these cultures, 0.1mL loop aliquot was streaked on 5% sheep blood agar and incubated under anaerobic conditions at 37°C for 24 hours. Colonies showing related characteristics of *C. perfringens* (aspect, color, and hemolysis) were submitted to Gram stain; colonies corresponding to Gram–positive bacilli were cultivated in CMB. After the incubation period, cultures were submitted to additional tests including catalase, lecithinase and gelatinase production, and glucose, lactose, and skimmed milk fermentation for species identification (Cowan, 1974). Strains identified as *C. perfringens* were then sub–cultivated in Triptose Yeast Extract Broth (TYB) and incubated under anaerobic conditions. Cultures were then centrifuged at 7.500 rpm for 15 min. at 4°C and cell–free culture supernatants were recovered. After that, 0.3 ml of broth culture supernatant and intestinal contents were injected into white mice (25 – 40 g) via intraperitoneal route and observed for either death or disease signs within three days.

C. Perfringens Toxins ELISA

Intestinal contents and broth cultures supernatant were tested for CPA, CPB, and ETX via a commercial capture ELISA kit (Bio–X, Diagnostics, Belgium), following the manufacturer's instructions.

RESULTS

Affected calves were almost found dead within 24 to 36 hours after the onset of clinical signs. Alternatively, others were found recumbent and semi–conscious, or still standing. Also, there were anorexia, signs of colic and dark clotted blood in the feces (melena) (Figure 1a). Moreover, the outstanding haematological finding in most cases was an often profound neutrophilia. *C. perfringens* was isolated from the intestinal contents. All strains were identified as *C. perfringens* that was based on colonial morphology, haemolysis on blood agar, Gram stain and biochemical characterization.There was no *A. fumigatus* or aflatoxins have been identified.



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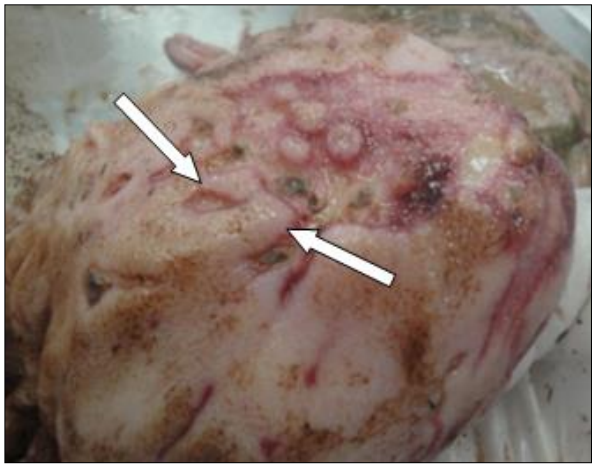

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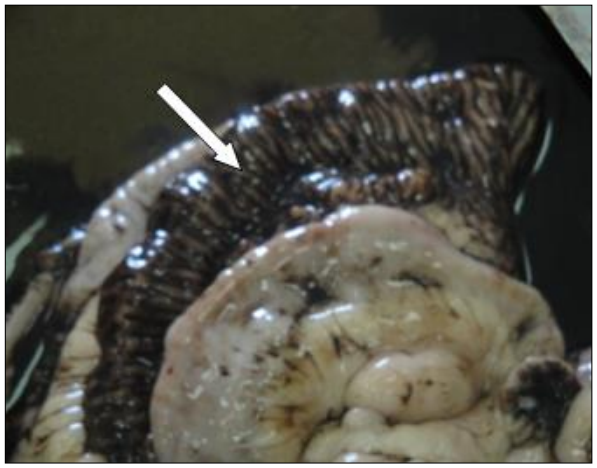

d

Figure 1: a– Dead cow; Note bloody stool (arrow); b–. Abomasum showing multiple ulcers (arrows); c– Small intestine distended with blackish blood (arrow); d– Ileum showing blackish mucosa (arrow)

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Toxin detection

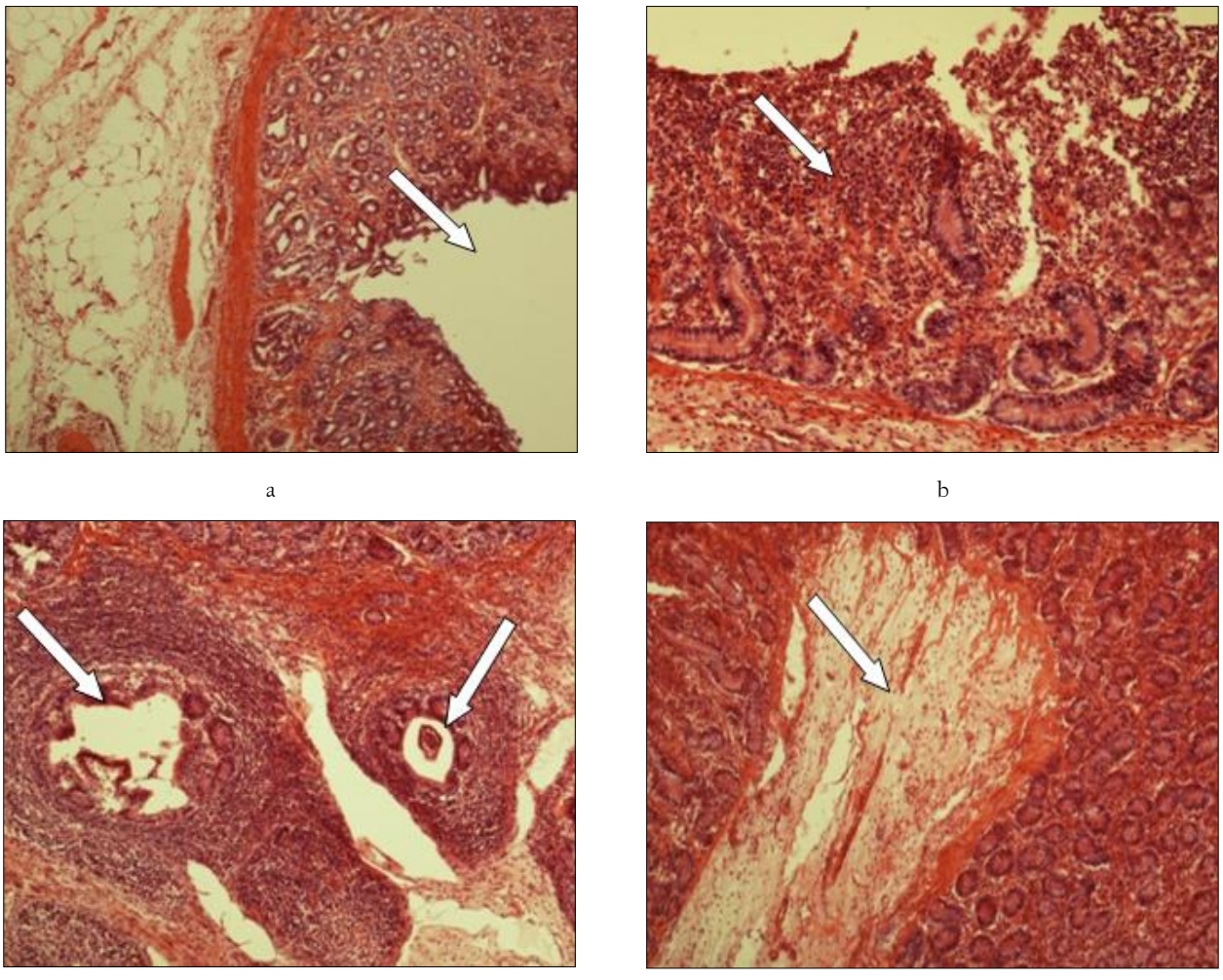
Toxin was detected in intestinal contents and broth culture supernatants, where all mice were died within 72 hours. A toxin was typed by using of an indirect ELISA assay. The results were positive for CPA and negative for CPB and ETX toxins. Hence, it was identified as *C. perfringens* type A.

Necropsy findings

The abomasum of most cases revealed multiple ulcers throughout the abomasal folds (Figure 1b).The small intestine contained either blackish blood or a large solid blood clot that obstructs the lumen (Figure 1c), and the mucosal surface of intestine appeared black in colour (Figure 1d). The aforementioned lesions were associated with congested and haemorrhagic mesenteric lymph nodes.

Microscopic lesions

The abomasal mucosa was denuded in more than one part and appeared as depressed areas (Figure 2a). The villi of jejnum and ileum appeared necrotic and heavy infiltrated by inflammatory cells (Figure 2b). The submucosa of ileum revealed a cluster of glands herniated into peyer's patches (colitis cystic profunda) (Figure 2c).The salient lesion in the small intestine was the presence of a characteristic submucosal oedema (Figure 2d).



a b c d

Figure 2: a– Abomasum. Note sloughed area (arrow); HE X200; b– Jejunum showing necrotic mucosa (arrow); HE X400; c– Ileum showing colitis cystic profunda (arrows); HE X200; d– Jejunum showing submuosal oedema (arrow); HE X200.

DISCUSSION

The present study describes a disease characterized by sudden death in calves. Typical gross lesions at necropsy and bacteriology along with toxin detection confirmed that *Colstridium perfringens* type A was incriminated in such condition. A final diagnosis was not only based solely on toxin detection, but also accompanied by pathological as well as microbiological findings (Francisco and Glenn, 2008). From pathological point of view, it is worth to find out colitis cystic profunda. This lesion may be a sequel to local damage to the muscularis mucosa (Jubb et al., 1993).

Colstridium perfringens type A is ubiquitous in the digestive tract of cattle and a hypothesis for the aetiology of *C. perfringens* overgrowth, is the overflow of finely ground carbohydrates from forestomach (Ewoldt and Anderson, 2005). This situation arises in association with the same factors which lead to sub–acute ruminal acidosis due to feeding on excess amounts of rapidly fermented carbohydrates or insufficient effective fiber

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(Gooden, 2003). Another explanation for the aetiology is sudden change in diet, *C perfringens* proliferates and produces potent toxins that act locally or are absorbed systemically (Niilo, 1980; Manteca 2002).

Clostridium perfringens type A produces CPA and can also produce several of the non–typing toxins, including CPE and CPB2 (Ceci et al., 2006; Brown et al., 2007). Information about pathogenesis of type A enteric infections in ruminants is minimal and often contradictory, but it is generally assumed that most clinical signs and lesions are due to the effects of CPA which is hemolytic, necrotizing, and potently lethal (Songer, 1996). *C. perfringens* type A also produces b2 toxin, which has a synergistic role with α toxin in the development of hemorrhagic lesions in the small intestinein cases of bovine enterotoxemia (Manteca et al., 2002) and in sheep and goats (Gkiourtzidis et al., 2001; Bueschel et al., 2003; Dray 2004,). In the present cases, the presence of b2 was not investigated. More studies are warranted to understand the role of b2 toxin in enterotoxemia cases caused by *C. perfringens* type A. In general, it is always possible to isolate *C. perfringens* type A from intestinal contents and therefore the detection of lethal toxins in intestinal contents is important for the diagnosis of enterotoxemia (Hakan et al., 2007).

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