ORIGINAL ARTICLES

Microbiological Studies On Enteritis Caused By *Clostridium Perfringens* Type A, In Sheep In Saudi Arabia

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**ABSTRACT**

*Clostridium perfringens* type A was isolated from 89 out of 121 sheep showing diarrhea, 7 (5.8%) sheep as a single infection and 82 sheep (67.7%) sheep as a mixed infection with coccidia. These sheep showed signs of enteritis and were suspected to have enterotoxaemia followed by sudden death during the period from May 2010 to August 2011. An experimental study was carried out on 3 groups of Guinea pigs, each group contained 2 infected animals and 1 control, (1st group inoculated I/M with the isolated *Clostridium perfringens* type A; 2nd group infected orally with *Eimeria* oocysts and 3rd group inoculated I/M with the isolated *Clostridium perfringens* type A and infected orally with *Eimeria* oocysts) for studying the pathogenicity of the isolated *Clostridium perfringens* type A as a single and mixed infections in the internal organs. The main characteristic histo-pathological findings in the small intestine of both sheep and Guinea pigs were destruction of the villi and edema. Intravascular haemolysis and haemorrhage were found in the intestinal mucosa and sub-mucosa. Whereas, the mesenteric lymph nodes were revealed follicular hyperplasia and the liver showed focal areas of necrobiotic changes. The results revealed that, most cases of enteritis were collected in winter season where the green fodder is available which was to be considered as a source of coccidial infection.

**Key words:** Microbiological studies - *Clostridium Perfringens* - Bacteriological studies - diarrhea - Enteritis - diarrhea in sheep

**Introduction**

Enterotoxaemia is a term used to describe disease caused by toxins produced by *Clostridium perfringens* within the intestine. *Clostridium perfringens* type A is an anaerobic spore forming rod that can exist for several months in soil after being discharged in the faeces and present in small numbers in the digestive tract of healthy animals causing enteric disease in sheep (Songer, 1996; Quinn et al, 2000 and Ahsani et al, 2011). *C. perfringens* type A produce α toxin. This toxin is a phosphor-lipase in nature which is lethal and necrotizing. It causes lysis and disrupting cell membranes leads to cell death. Also causes increase vascular permeability through endothelial damage and necrosis at the tips of villi of intestine (Feldman, 2000). Epsilon toxin binds to receptors in the luminal surface of the vascular endothelium, producing degeneration of vascular endothelial cells, allows ion leakage and alteration of fluids dynamics (McDonel, 1986 and Jones et al., 1997).

Al Mashat and Taylor (1983) isolated (*clostridia perfringens* type A and *E. coli* from the necrotic haemorrhagic small intestine of ewe that died after developing diarrhea. The intestinal mucosa showed intense fluid secretion and inflammatory cellular accumulations of macrophages, neutrophils, lymphocytes and mast cells (Rocha et al, 1999). Coccidiosis is a disease of major economic importance because the coccidia are considered an essential predisposing factor for bacterial pathogen infection (Nibal, 1999). Intestine suffered from coccidiosis showed fluid distention, hyperaemia and pin point white necrotic foci grossly.

Microscopically, it revealed necrosis of villus or crypt epithelium, hyperaemia and moderate inflammatory cell infiltration (Thomson, 1995).

Although *Clostridium perfringens* type A infection represents one of the most serious problems affecting sheep due to severe economic losses as a result of sudden death of sheep, its pathogenicity in animals is not well studied.

The purpose of the work under investigation to throw a light on the interaction between *Clostridium perfringens* type A infection and coccidial infection in sheep in Saudi Arabia.

**Material And Methods**

A total of 121 sheep aging from 6-12 months old, belonging to 5 different farms in Taif governorate, were used in this study. These sheep showed signs of enteritis and were suspected to have enterotoxaemia followed by sudden death during the period from May 2010 to August 2011. Samples were collected from small intestine.
of each sheep to fulfill bacteriological and parasitological examinations. Meanwhile, tissue specimens were collected from small intestine, mesenteric lymph nodes and liver for histo-pathological examinations after complete post-mortem examination.

Experimental study:

Nine healthy Guinea pigs with average weight 350 - 400 gm were used in this work to study the pathogenicity of the isolated *Clostridium perfringens* type A (single and mixed infections) in the internal organs. The Guinea pigs were classified into 3 groups; each group contained 3 animals (2 infected and 1 for control).

First group included 2 Guinea pigs inoculated intramuscularly with 0.5 ml of 24 hours cooked meat broth culture mixed with 0.5 ml of 5% calcium chloride solution (Willis, 1964).

Second group included 2 Guinea pigs infected orally with 1 ml of potassium dichromate containing 2500 *Eimeria* oocysts (Malaka, 1996).

Third group included 2 Guinea pigs inoculated I/M with 0.5 ml of 24 hours cooked meat broth culture mixed with 0.5 ml of 5% calcium chloride solution and infected orally with 1 ml of potassium dichromate containing 2500 *Eimeria* oocysts.

Each group was housed individually and the Guinea pigs were kept under observation for 72 hours or until death.

After death, post-mortem examination was carried out and samples and tissue specimens from each Guinea pig were collected for bacteriological (re-isolation of *Clostridium perfringens* type A), parasitological (redetection of coccidia) and histo-pathological examinations.

I- Bacteriological examinations:

1- Sampling:

Congested portions of the small intestine (sheep and G. pigs) with their contents were collected directly after death and ligatured in both ends. They were put in sterile plastic bags under complete aseptic condition and transferred in an ice box as soon as possible to the laboratory.

2- Isolation and identification of *Clostridium perfringens* type A:-

Intestinal contents were subjected to isolation and identification of *Clostridium perfringens* according to Cruickshank *et al*, (1975) and typing of toxigenic strains was carried out by dermo-necrotic test in Guinea pigs (Oakely and Warrack, 1953 and Quinn *et al*, 2002).

II- Parasitological examination:

Detection and identification of *coccidia* were done on the intestinal contents of dead sheep and stool of Guinea pigs according to Soulsby (1986).

III- Histopathological examinations:

Specimens from small intestine, mesenteric lymph nodes and liver of dead sheep and Guinea pigs were collected and immersed in 10% formal saline. Fixed specimens processed by paraffin imbedding technique then sectioned at 4 – 6 µ thickness and stained with H&E (Bancroft and Stevens, 1990).

Results

I- Bacteriological results:

*Clostridium perfringens* could be isolated from the collected samples of 89 out of 121 diarrheic sheep: 7 (5.8%) sheep as a single infection and 82 (67.7%) sheep as a mixed infection with *coccidia*.

Estimation of toxins produced by the toxigenic strains of *Clostridium perfringens* revealed the identification of *Clostridium perfringens* type A. (Fig. 1)

II- Parasitological result:

*Eimeria ovinoidea* oocysts could be detected in 82 (67.7%) sheep as a mixed infection with *Clostridium perfringens* type A.
Table I: Showing the number of animals revealed single and mixed infections of *Clostridium perfringens* type A and their relations to season.

<table>
<thead>
<tr>
<th></th>
<th>Single infection 7 sheep (5.8%)</th>
<th>Mixed infection 82 sheep (67.7%)</th>
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<tr>
<td></td>
<td>Winter</td>
<td>Summer</td>
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<tr>
<td>No.</td>
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<td>4</td>
<td>57.1</td>
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III- Pathological results:

**Sheep:**

*A- Sheep revealed single infection with Clostridium perfringens type A (7 sheep):-

• Small intestine:

  **Gross findings:**

  Intestine appeared flaccid and distended with gases and their contents revealed watery fluid which usually haemorrhagic focal or diffuse. Congestion and necrosis were noticed in the intestinal mucosa. (Fig. 2) Gross lesions appeared mostly in jejunum and ileum.

  **Histo-pathological findings:**

  Intestinal mucosa revealed wide spread areas of coagulative necrosis involving some glands and most of the villi appeared completely destructed and replaced by tissue debris and mononuclear inflammatory cells (Fig. 3). Meanwhile, severe congestion of the blood capillaries and wide dilatation of the lymphatics were seen in some villi. Lamina propria showed intravascular haemolysis and mild edema (Fig. 4).

*B- Sheep revealed mixed infection with Clostridium perfringens type A and coccidia (82 sheep):-

Pathological findings observed in the small intestine of these sheep were similar, to great extent, to those observed in single infection of *Clostridium perfringens* type A. In addition, coccidial oocysts were seen mixed with the necrotic tissue at tips of the villi of intestine (Fig. 5).

1- Guinea pigs:

**First group (inoculated with isolated Clostridium perfringens type A):**

• **Small intestine:**

  **Gross findings:**

  Intestinal mucosa covered by haemorrhagic fluid and revealed few erosions and necrosis which appeared markedly in jejunum and ileum. Edema of the intestinal wall was also seen.

  **Histopathological findings:**

  Intestinal mucosa appeared destructed and necrosed. Lamina propria and sub-mucosa showed moderate edema and infiltration of lymphocytes, macrophages and plasma cells. Congestion accompanied by characteristic intravascular haemolysis was evident in sub-mucosal blood vessels. (Fig. 6)

**Second group (infected with Eimeria oocysts):**

• **Small intestine:**

  **Gross findings:**
Mucosa of the Jejunum and ileum revealed congestion, erosion and bleeding areas. Pin point white foci were visible from both mucosal and serosal surfaces.

**Histopathological findings:**

Intestinal mucosa showed degeneration and exfoliation of the enterocytes and focal areas of coagulative necrosis at tips of the villi. Congestion and mononuclear cell infiltration (lymphocytes, macrophages and eosinophils) were evident in the lamina propria of small intestine. In addition, the glandular epithelium appeared hyperplastic and revealed Eimeria stages.

**Third group (infected with isolated Clostridium perfringens type A and Eimeria oocysts):**

- **Small intestine:**

  **Gross findings:**

  Distension of the small intestine with yellowish fluid which appeared tinged with blood in Jejunum and ileum. Intestinal mucosa showed diffuse congestion and ecchymotic haemorrhages.

  **Histopathological findings:**

  Marked villus atrophy and necrosis associated with exfoliation of enterocytes were predominant lesions observed in the intestinal mucosa. Exfoliated enterocytes and intestinal crypts showed *Eimeria* stages which also occupying most of the glandular epithelium. Sub-epithelial haemorrhages together with diffuse coagulative necrosis and inflammatory cell aggregations consisting of lymphocytes, macrophages and eosinophils were seen in the lamina propria of jejunum and ileum. Meanwhile, diffuse edema and intravascular haemolysis were observed in mucosa and sub-mucosa. Glandular epithelium appeared hyperplastic mostly in duodenum.

**Fig. 1:** *Clostridium perfringens* cultivated on blood agar. Surrounding the colonies, an inner, complete zone of hemolysis is caused by perfringolysin O and the less complete outer zone is caused by Cl. perfringens alpha toxin.

**Fig. 2:** Small intestine of sheep with hemorrhagic enteritis due to *Clostridium perfringens* type A. The intestine is distended with gas and hemorrhagic fluid.
Fig. 3: Small intestine of sheep revealed single infection with *Cl. perfringens* type A showing intense infiltration of mononuclear inflammatory cells and moderate neutrophilic infiltration in the lamina propria (H&E; X100).

Fig. 4: Small intestine of sheep revealed single infection with *Cl. perfringens* type A showing destruction with necrosis of the villi and edema in the lamina propria (H&E; X100).

Fig. 5: Small intestine of sheep revealed mixed infection with *Cl. perfringens* type A and *coccidia* showing *Eimeria* stages in the epithelium of intestinal glands (H&E; X400).
Fig. 6: Intestine of G. pig inoculated with isolated Cl. perfringens type A showing destruction and necrosis of the mucosa; and intravascular haemolysis (H&E; X100).

Discussion:

Members of genus Clostridium were known to be extraordinarily in their natural habitats and when introduced to animal hosts, a few produce acute and often fatal disease which mediated by one or more of the many toxic protein produced by these organisms (Nibal, 1999).

The present work showed that Clostridium perfringens type A could be isolated from 89 (73.5%) out of 121 sheep revealed signs of enteritis followed by sudden death, meanwhile detection of other bacteria or parasites was unsuccessful in other sheep (32). This high percentage of isolation indicates that Clostridium perfringens type A represents one of the most important pathogens causing enteritis or sudden death in sheep particularly if it mixed with coccidial infection as reported by AI-mashat and Taylor (1983) and El-drissi et al. (1992). Sheep revealed isolation of Clostridium perfringens type A (89) included 7 (5.8%) sheep with single infection and 82 (67.7%) sheep with mixed infection with coccidia. Single infection agreed to some extent with Mills et al, (1990); Hosein et al, (1995) and mixed infection agreed to large extent with Nibal (1999).

Our study also revealed somewhat relationship between season and percentage of enterotoxaemia in sheep where the winter (green season) showed high percentage of infection with enterotoxaemia (57.1% as a single infection and 93.9% as a mixed infection with coccidia). Similar observations were recorded by Ballal (1990), Tooloei and Masodie (2008) and Yakhchali, M. and E. Golami, (2008).

High percentage of mixed infection (93.9%) in winter confirms the suggestion that coccidia were considered an essential predisposing factor for bacterial pathogen infection. Winter season has high incidence of coccidial infection resulting in massive widespread mucosal damage and haemorrhage. Thus, enhancing the growth and multiplication of commensal Clostridium perfringens to become vegetative and enterotoxigenic releasing powerful lethal toxins (Hungerford, 1990 and Komoriya et al, 2007). The latter is acting by increasing permeability of the intestinal mucous membrane, hence facilitating passage of the toxins and organism into blood stream leading to hypoxaemia which favours growth and multiplication of the organism. Similar view was given by Martin et al. (1991) and Shalaby (1996). In addition, Thomson (1995) and Rubin et al, (1998) stated that, the condition of mal-absorption due to coccidial invasion activate the toxin produced by Clostridium perfringens. This is achieved by the action of pancreatic enzyme trypsin which renders inactive endo-toxins into active toxigenic one.

Distension of intestine with gases as well as with thin watery, mucoid or bloody content observed through postmortem examination come in accordance with Robbin et al (1997) and Mansour (2004).

Putrefaction is well known to be accompanied by excess gas formation in affected intestine. The toxins produced by Clostridium perfringens combined with the intestinal cells leading to secretion of body fluids and electrolytes into the gut causing deregulation of ion pump mechanism which result in severe diarrhea. These results are in concomitant with those described by Blood and Radostitis (1989) and Hosein (2003). Moreover, Davis et al. (1998) recorded that; diarrhea was attributed to increased acetyl choline in inflamed intestine where the cholinergic enervation of the intestine can undergo rapid long-lasting alteration during inflammation.

The relative alkalinity of jejunum and ileum which is favorable for clostridial growth and population may give an explanation for the severity of lesions observed in these segments of small intestine. Benauoda (1985); Mahmoud (1991) and Shalaby (1996) gave a supportive opinion for such result. The most predominant histopathological alterations noticed in this study were destruction and/or necrosis at tips of the villi and edema, intravascular haemolysis, haemorrhage, inflammatory cell infiltrations in the intestinal mucosa and/or sub-
mucosa. These findings come in agreement with the observations of Rocha et al. (1999); Hosein (2003) and Mansour (2004). Circulating powerful toxins produced by *Clostridium perfringens* type A led to intravascular haemolysis which was seen in the examined small intestine; and increased permeability of the blood vessels. This suggestion was supported by the reports given by Jones et al., (1983); Jubb et al., (1994) and Venkova et al., (1999).

On the other hand, Blood et al. (1983); Shalaby (1996) and Rubin et al., (1998) reported that, absorbed toxins cause haemolysis and sub-epithelial edema where ischemia and haemolysis result in necrosis and/or sloughing of the tips of intestinal villi. As a result of edema, necrosis and shedding as well as haemorrhages from damaged villi. The intestinal contents appeared haemorrhagic. The latter are considered a good medium for a more enhancement of clostridial multiplication (commensal become enterotoxigenic), hence more invasion and tissue destruction. This hypothesis was given by Nibal (1999).

The obtained results revealed that, clotridial toxins play an essential role in the infiltration of inflammatory cells (macrophages, lymphocytes and sometimes neutrophils) in the small intestine. Such result coincided with that of Rocha et al., (1999) who demonstrated the importance of toxins A & B in the pathogenesis of inflammatory diarrhea induced by *Clostridium* spp. They recorded that, toxin B stimulates the synthesis of potent inflammatory mediators by monocytes and macrophages and the effect provoked by toxin A on the intestinal mucosa characterized by intestinal fluid secretion as well as inflammatory cell accumulation such as macrophages, lymphocytes and neutrophils.

The prominent microscopical lesions of intestinal coccidiosis observed in the present data were as similar findings that noticed by Cheville (1983); Jubb et al., (1994) and Mansour (2004). Hyperplastic activity of the intestinal glands and crypts could be regarded as a reparative mechanism in response to the rupture and desquamation of parasitized epithelial cells by the developing stages of *Eimeria*. This explanation comes in harmony with the opinion of Ovington et al., (1995).

**Conclusion:**

*The results mentioned above proved that:*

- *Clostridium perfringens* type A represents one of the common pathogens recovered from sheep suffered from enteritis.
- Mixed infection (*Clostridium perfringens* type A & coccidia) seems to be the rule rather than exception and represents the most prevalent form of enteritis.

**Recommendations:**

1. Vaccination of dams at last stage of parturition against *Clostridium perfringens* type A for control of enterotoxaemia in sheep in enzootic areas.
2. Periodical examinations for calves detected positive for coccidia and it should not be ignored because *coccidia* is a contributing factor causing sudden death due to enterotoxaemia in sheep.
3. Proper hygienic measures must be applied in the sheep farms.

**Acknowledgement**

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**References**


