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**AETIOPATHOLOGY OF ENTERITIS IN SHEEP  
CAUSED BY ANAEROBIC AND PARASITIC AGENTS:  
A LIGHT AND SCANNING ELECTRON  
MICROSCOPIC STUDY  
(With 3 Tables and 10 Figures)**

By

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دراسات باثولوجية علي الالتهاب المعوي في الخراف  
الناتج عن الإصابة بالبكتريا اللاهوائية والطفيليات المعوية،  
دراسة بالميكروسكوب الضوئي والإلكتروني الماسح

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أجريت الدراسة علي مائة خروف مصابة بالإسهال من بين ثلاثمائة وخمسون خروفا تم فحصها في الحجر البيطري الخاص بمجزر الجيزة تراوحت أعمارها ما بين ٤ إلى ١٨ شهرا. فحصت الأمعاء الدقيقة بكتريولوجيا وطفيليا وهستوباثولوجيا. وقد وجد أن ميكروب الكلوستريديوم بيرفرينجنس نموذج ١ هو الأكثر شيوعا وجاءت النماذج ب، ج، د تالية له من حيث الانتشار. هذا وقد أوضحت الدراسة وجود علاقة وطيدة بين الإصابة بطفيل الكوكسيديا وظهور النشاط السام لميكروب الكلوستريديوم بيرفرينجنس. أوضحت الدراسة الباثولوجية وجود تغيرات بالأمعاء اشتملت علي أودوما تحت الطبقة المبطننة للأمعاء والتي ظهرت كثرات ثلاثية عند الفحص بالميكروسكوب الإلكتروني الماسح وكذلك ظهر تركز بخلايا الطبقة السطحية لجدار الأمعاء بالإضافة إلى بعض التغيرات بالأوعية الدموية والليمفاوية.

**SUMMARY**

The present work was conducted on 350 slaughtered sheep of which 100 cases showing diarrhea. Their ages ranged from 4-18 months. The intestinal tract was subjected to thorough bacteriological, parasitological and histopathological examination. Toxogenic *Clostridium perfringens* were identified in 37 cases of which 30 cases were combined with coccidial species. The histopathological changes of such cases revealed superficial mucosal necrosis and marked proprial oedema. The sequential examination of the small intestine by scanning electron

microscopy (SEM) demonstrated the presence of epithelial blebs of oedema and clostridial bacilli adherent to the enterocytes. Coccidia represented the highest percentage (57%) of infestation among the intestinal parasites. It was also considered an important predisposing cause for *Clostridium perfringens* proliferation and invasion.

**Key words:** *Enteritis, sheep, anaerobic & parasitic agents, electron microscopic study.*

## INTRODUCTION

Enteritis is a major problem facing sheep production. It represented about 20% of all Gastrointestinal Tract (GIT) disorders (Srivastava *et al.*, 1985). Intensive investigations have long been indicated that there may not be a single etiology of enteritis but rather the cause is complex and usually involves an interplay between enteropathogenic bacteria, viruses and parasites; immunity of the animal and the environmental factors. Enteric clostridial diseases were regarded as the major cause of death in sheep due to enteritis (Sconning and Sagartz, 1986 and Urban *et al.*, 1987). One or other of the five-toxogenic types of *Clostridium perfringens* causes the diseases. In spite of the fact that *Clostridium perfringens* is a normal inhabitant of GIT, certain predisposing factors are needed for the production of the disease such as the decrease in the intestinal motility in case of over-eating or sudden change from poor to rich diet. These factors participate in the pathogenesis of clostridium proliferation with production of localized enteric infection and/or toxemia (Hungerford, 1990). The intestinal lesions produced by *Clostridium perfringens* varied from being catarrhal gastroenteritis (Urban *et al.*, 1987) to severe haemorrhagic enteritis with necrosis and sloughing of mucosal epithelium of jejunum, ileum and part of duodenum (Nillo, 1987).

Gastrointestinal parasites are common causes of enteritis in sheep less than 2 years old (Belot and Pangu, 1986). In Egypt, Aly (1990) showed that the infestation with coccidial protozoon in sheep represented the highest percentage (24.56%) of infestation among all parasites causing diarrhea.

The purpose of the work under investigation was to throw a light on the interaction between enteric anaerobic infection and parasitic infestation in sheep with regard to the intestinal pathological alterations.

## **MATERIAL AND METHODS**

### **Animals:**

Three hundred and fifty sheep of 4-18 months age were examined in the quarantine of Giza Slaughter house during the period from January 1997 till December 1998. One hundred cases showed signs of diarrhea. The intestinal tract of the diarrheic cases was subjected to thorough postmortem examination. The intestinal contents and tissue specimens were collected from duodenum, jejunum, ileum and mesenteric lymph nodes to fulfill bacteriological, parasitological and histopathological examinations.

### **Bacteriological examination:**

Portions of the small intestine with their contents were ligated at both ends and kept in sterile labeled plastic bags.

The intestinal content was cultured on cooked meat media for isolation and identification of anaerobes according to Cruickshank *et al.* (1975).

The toxogenic strains of *Clostridium perfringens* were identified according to Bullen (1952) by using peptone media. Typing of toxogenic strains was done by dermonecrotic test in guinea pigs (Oakely and Warrack, 1953).

### **Parasitological examination:**

The intestinal helminthes were collected from the intestinal contents and identified (Soulsby, 1986). Coccidial oocysts were identified from the intestinal contents and mucosal scraping and the examined sample was considered positive when it contained at least 5000 oocyst/gm (Soulsby, 1986).

### **Histopathological examination:**

For light microscopy, tissue specimens from the successive parts of small intestine and mesenteric lymph nodes were fixed in 10% neutral buffered formalin, processed by paraffin embedding technique, then sectioned at 4-6 $\mu$  thickness and stained with H&E.

For scanning electron microscopy (SEM), the specimens were fixed in 1% osmium tetroxide for 2hs, washed in cacodylate buffer, dehydrated by ascending grades of alcohol and then washed with amyloacetate 2 changes for 2 days. The specimens then were prepared for coating with gold and examined by GEOL 540 LV scanning electron microscope.

## RESULTS

### Bacteriological and parasitological findings:

The results of bacteriological and parasitological examination were summarized in Tables 1, 2 and 3.

**Table 1:** Showing serotyping of anaerobic bacteria isolated from enteric sheep and their toxins.

Types of isolates	Number of infected cases	Types of toxins				
		A	B	C	D	E
Toxogenic Cl. Perfringens	37	20	12	3	2	-
Non toxogenic Cl. Perfringens	5					
Peptostreptococcus anaerobovis	5					

**Table 2:** Showing the incidence of gastrointestinal parasites recovered from enteric sheep.

Parasites	Species	No. of infested cases
Nematodes	Trichostrongylus	23
	Nematodirus	11
	Cooperia	9
Total cases		43
Cestodes	Monizia expansa	14
	Monizia bendeni	4
	Avitellina sp.	7
Total cases		25
Protozoa	Eimeria intricata, Eimeria arloingi, Eimeria ninakohlyakinovae, Eimeria fauri, Eimeria crandolis, Eimeria ahasata and Eimeria parva	57 (more than one sp. were isolated from each case)

**Table 3:** Showing the incidence of Clostridium perfringens and associated parasitic agents recovered from enteric sheep.

Type of pathogens	Number of infected cases
Toxogenic Clostridium perfringens and Coccidia	30
Toxogenic Clostridium perfringens and Trichostrongylus	5
Toxogenic Clostridium perfringens and Avitellina	2

### Pathological findings:

Gross lesions of the intestine were variable and appeared mostly in jejunum and ileum. The affected segments were hyperemic and

distended with watery content or gases. The mucosa showed patchy or diffuse congestion and in severe cases petechial or ecchymotic haemorrhage. In some cases, numerous grayish or yellowish foci were visible either on serosal and mucosal surfaces. The mesenteric lymph nodes of the corresponding affected region were swollen and congested.

In most affected cases with *Clostridium perfringens*, the microscopic examination revealed superficial mucosal necrosis with prominent separation of the covering epithelium from the underlining expanded lamina propria (Fig. 1). In the duodenum, the lesions were mild in nature and were manifested by necrosis of the villous tips with mononuclear cell infiltration in the lamina propria. The histopathological changes in the jejunum varied from being moderate exfoliation of the superficial enterocytes with marked congestion of the proprial capillaries to severe coagulative necrosis of the superficial one quarter to one third of the jejunal mucosa with necrotic material get admixed with extravasated blood (Fig. 2). In some cases, coccidial oocysts could be detected within the necrotic debris in the intestinal lumen (Fig. 3).

SEM of the jejunum revealed presence of marked subepithelial oedema separating the covering epithelium from the underlying structures (Fig. 4). Overview of the necrotic surface showed adherent rod-shaped bacillus with marked cellular exudate (Fig. 5). The ileal changes were characterized by massive necrosis of whole length of some villi associated with marked villous atrophy and cryptal hyperplasia. In most cases the cryptal epithelium were occupied by different coccidial gametes. The lamina propria was mostly expanded with dilated lymphatics and heavy inflammatory cell infiltration mainly mononuclear cells (Fig. 6).

SEM of the ileum demonstrated the process of apoptosis affecting some enterocytes before their exfoliation from the surface (Fig. 7). It also exhibited the presence of extensive epithelial blebs of edema (Fig. 8) as a common finding in clostridium infected cases.

In severe cases, the complete destruction of some villi resulted in direct exposure of intestinal crypts towards the lumen (Fig. 9).

The intestinal lesions in case of parasitic infestation were mostly manifested by marked villous atrophy, mucosal erosion at the site of attachment of the worm with prominent inflammatory cell infiltration in lamina propria mainly eosinophiles and globule leukocytes (Fig. 10).

In nematode infestation, cross-section of migratory larvae could be detected in the mucosa and submucosa of the small intestine.

## DISCUSSION

The present study denoted that clostridium infection is still considered the most important and wide spread pathogen among the bacterial causes of enteritis in sheep as their incidence reached 37%. This incidence is significantly low when compared with the results of Mahmoud (1991), 66.5%. Such variation could be attributed to the number and source of animals examined, nature of food habits and the hygienic measures. On the other hand, *Clostridium perfringens* was isolated from sheep died with enterotoxaemia in a percentage of 39% (Debeljak *et al.*, 1991) and 33.6% (EL Edressi *et al.*, 1992). In the current work, *Clostridium perfringens* type A was the most prevalent one followed by type B, C and D., a result which was in accordance with that mentioned by Mahmoud (1996). The superficial mucosal necrosis and prominent proprial oedema seen in the small intestine might be due to the effect of enterotoxins produced by *Clostridium perfringens* type A, C and D. Jubb *et al.* (1994), in case of X colitis in horse, mentioned similar explanation to the effect of enterotoxine produced by type A clostridium.

The SEM supported the light microscopic observations about the presence of epithelial blebs of edema affecting scattered cells among the mucosal covering. Such observation might indicated a direct action upon the enterocyte, as the blebs of edema resulted from the interference with the active sodium-potassium balance in the cytoplasm leading to influx of sodium together with water into affected cells (Ballal, 1990). The subepithelial edema together with the hyperemia of the mucosal blood vessels and lymphatic dilation declared the action of exotoxin released from *Cl. perfringens* on the endothelial lining of the vessels.

In the present study, *Cl. perfringens* was isolated mainly from cases with intestinal coccidiosis. This combination was found in 30 cases over the 37 positive cases for toxogenic *Cl. perfringens*. This finding denoted that coccidial infestation is considered an important predisposing factor for *Cl. perfringens* invasion. The severe tissue destruction caused by coccidia provided a good media for *Cl. perfringens* proliferation and the elaboration of enterotoxin. The latter was released only on lytic cells as reported by Jubb *et al.* (1994). The evaluation of the diarrheic cases reported here, revealed the absence of toxemia although *Cl. perfringens* was isolated from such cases. This could be attributed to the age of the animals, (the small ages were more susceptible), the amount of toxin present in the intestinal content, nature

of the food of the animals, and the presence of circulating antitoxins. Such factors might help the absence of toxemia in such diarrheic cases.

## REFERENCES

- Aly, U.A.H. (1990):* Studies on parasitic and bacterial diarrhea in sheep. M.V.Sc. Thesis, Faculty of Veterinary Medicine, Assuit University.
- Ballal, A.I. (1990):* Light and transmission and scanning electron microscopical investigations of the intestinal tract with special reference to enteritis in calves. M.V.Sc. Thesis, Fac. Vet. Med. Assuit University.
- Belot, R.J. and Pangui, J.L. (1986):* Observation on the fertility of gastrointestinal strongyles of sheep in limited study at Dakar abattoirs: Preliminary remarks and parasitic nodules. *Rev. Med. Vet. Parasit.*, 137(7), 533-536.
- Bullen, J.J. (1952):* Enterotoxaemia in sheep by clostridium welchii type D in the alimentary tract of sheep. *J. Pathol. Bacteriol.*, 64, 201-206.
- Cruickshank, R.D.; Duguid, J.P.; Marmion, P.B. and Swain, R.H.A. (1975):* Medical microbiology. Vol. II, 12<sup>th</sup>. Ed. Livingstone. Edinburgh, London and New York.
- Debeljak, Z.; Plavisc, Z. and Zarkovic, A. (1991):* Anaerobic infections in sheep and their importance in the kraljevo region of Serbia. *Veterinarski-Glasnik*, 45 (8), 537-540.
- EL Ederissi, A.H.; Ward, G.E.; Johnson, D.W.; Benkirane, A. and Fassi-Feri, M.M. (1992):* Bacteriological investigation of sudden sheep mortality in Morocco. *Prenet Vet. Med.* 12 (1/2) 35-46.
- Hungerford, T.J. (1990):* Diseases of livestock. 9<sup>th</sup>. Ed. Australia by Macarthur Press Sales Pty Limited.
- Jubb, K.V.F.; Kennedy, P.G. and Palmer, N. (1994):* Pathology of domestic animals. Academic Press, Carlando, Florida.
- Mahmoud, B.S. (1991):* Isolation and identification of clostridia among apparently healthy slaughtered sheep and goat. M.V.Sc. Thesis, Fac. Vet. Med. Cairo Univ.
- Mahmoud, F.A. (1996):* Microbiological studies on obligatory anaerobic microorganism causing diseases in Egyptian sheep and goat. Ph.D. Thesis, Fac. Vet. Med. Cairo Univ.

- Nillo, L. (1987):* Toxogenic characteristic of cl. *Perfringens* type in enterotoxaemia of domestic animals. *Canadian J. Of Vet. Res.* 51 (2), 224-228.
- Oakley, C.L. and Warrack, G.H. (1953):* Cytokines and immunological control of *Eimeria* spp. *Int. J. Parasitol.* Nov. 25 (11), 1331-1351.
- Sconing, P. and Sagartz, J. (1986):* Lamb mortality in small confined sheep flock. *Modern Vet. Prsct.* 67(1), 20-23.
- Soulsby, E.J.L. (1986):* Helminthes, arthropods and protozoa of domestic animals. 7<sup>th</sup>. Ed. ELBS, London.
- Srivastava, A.K.; Patil, V.K. and More, B.K. (1985):* The weak immunogenicity of *fusobactrium necrophorum*. *Int. Vet. J.*, 62 (1), 935-939.
- Urban, V.P.; Shirobokova, M.M.; Semenkov, I.D.; Konopatov, Y.U. and Akil'zhanov, R.R. (1987):* Mixrd infection in sheep. *Doklody Vesesyuzoni Akademi Sel'skokhov Yaistvennykn Nauk*, 6, 35-37.

### LEGEND OF FIGURES

- Fig. 1:** Jejunum showing subepithelial edema with separation of covering epithelium. H&E, X 66.
- Fig. 2:** Jejunum showing diffuse coagulative necrosis of the superficial mucosa. Notice, the extravasated blood with the necrotic debris. H&E, X 66.
- Fig. 3:** Jejunum presented coccidial oocyst on the surface of necrotic mucosa. H&E, X 132.
- Fig. 4:** SEM graph of jejunum showing superficial necrosis of villous tips. Notice, the exposed hyperemic proprial capillaries. X 3500.
- Fig. 5:** SEM graph of jejunum showing rod shape bacillus adhered to the necrotic surface and associated with cellular exudate. X 5000.
- Fig. 6:** Ileum showing expanded lamina propria with inflammatory cells and dilated lymphatics. H&E, X 66.
- Fig. 7:** SEM graph of ileum demonstrating apoptic enterocytes. X 3500.
- Fig. 8:** SEM graph of ileum showing extensive blebs of edema and exfoliated enterocytes. X 1500.
- Fig. 9:** SEM graph of ileum showing necrosis of the whole length of villi with exposure of crypt toward the lumen. X 1000.
- Fig. 10:** Jejunum showing cestode in the lumen, necrosis and atrophy of the intestinal villi. H&E, X 13.2









