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BACTERIOLOGICAL AND PATHOLOGICAL STUDIES ON ENTERIC ORGANISMS WITH SPECIAL REFERENCE TO *SALMONELLA* AMONG SLAUGHTERED COWS AND BUFFALOES IN ISMAILIA GOVERNORATE

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Abstract

Bacteriological and pathological studies were carried out on 500 animals (336 cows and 164 buffaloes) slaughtered in two abattoirs at Ismailia Governorate, Egypt. From these animals 1500 proper samples were taken from small intestine, mesenteric lymph nodes and liver. The bacteriological examinations of these organs revealed that the total average percent of enteric bacterial isolates in cows was 66.6% and in buffaloes was 59.1%. The bacterial isolates were identified as *E. coli*, *Salmonella*, *Enterobacter*, *Proteus*, *Klebsiella*, *Citrobacter*, *Yersenia* and *Edwardsiella*. The percentage of these infections varied in both cows and buffaloes. The isolated *Salmonella spp.* were identified as *Salmonella enteritidis* and *S. cerro* with a percentage of infection in cows 13.98% and 7.47%, while, in buffaloes it was 10.4% and 3.65%, respectively. *Salmonella enteritidis* phage type 4 was diagnosed in both cows and buffalo *Salmonella*. Most of the enteric isolates did not influence the gross and histological structure of the examined organs. However, in *E.coli*, *Salmonella spp.* and *Yersinia* infections, the pathological changes in intestine, mesenteric lymph nodes and liver were pronounced and differed in their severity according to the type of infection. These changes were illustrated and discussed.

INTRODUCTION

Enteric organisms are world wide in distribution. There are both potentially pathogenic and non-pathogenic species. Bacteria belonging to the family enterobacteriaceae usually cause gastroenteritis in animals, which is always considered the major problem for the farmer and the veterinarians even under traditional farm conditions. Gastroenteritis is responsible for the morbidities and mortalities of the animals. The economic losses are not only due to sickness and death but also to medical costs for the bad health and poor growth condition of diseased animals.

Causes of enteritis are variable and differed according to species, breed and the age of animal. The state of nutrition and environmental condition would play a major role in the disease (El-Hamamy *et al.*, 1999). Ali *et al.* (1996), El-Hamamy *et al.*

(1999), Taha (1999) and Abd-Alla *et al.* (2000) recorded that *E. coli*, *Proteus*, *Salmonella* and *Enterobacter* were the most prevalent microorganisms causing enteritis.

El- Hamamy *et al.* (1999), in their studies on cow calves belonging to Salhya dairy farm at Ismailia Governorate stated that the bacteriological examination of diarrhoeic calves revealed that the predominant isolates were *E.coli* (52.5%), *Enterobacter aerogense* (15%), *Proteus vulgaris* (12.5%), *Salmonella spp.* (5%), *E.coli* and *Proteus spp.* (10%) and *E. coli* and *Enterobacter* (5%).

Jubb *et al.* (1993) recorded that the diarrhoea produced by enterotoxigenic *E. coli* is accompained by relatively minor microscopic evidence of inflammation and by little or no architectural change in the intestinal muscosa. However, where bacterial colonization of the surface of enterocytes is heavy, atrophy of the villi may be present. Transmigration of neutrophils from lamina propia to lumen is present in colonized areas of gut especially in the vicinity of the dams over Payer's patches. The problem of salmonellosis in calves is important from the economical point of view as cows and buffaloes of all ages are susceptible to infection with various *Salmonella* serotypes. In adult animals, salmonellosis is usually sporadic, and causes great losses (Farid *et al.*, 1979). Infected animal that recover spontaneously or after treatment commonly harbour the organism in their gut. These animals are called *Salmonella* carriers which disseminate the organism intermittently in their faeces. Infection with *Salmonella* may or may not lead to fatal salmonllosis, a disease that remains localized in the gastrointestinal tract as gastroenteritis, or becomes generalized as septicemia and affects several organs (Ekperigin and Nagaraja, 1998). Enteritis results in shortening of villi, degeneration of enterocytes, increased emptying of goblet cells and a neutrophilic reaction in the lamina propria, accompanied by transepithelial migration of neutrophils into the lumen. Early in the course of enteritis, thrombi are visible in the vessels in the lamina propria and later, damage to the vessel walls is associated with the thrombi (Gyles and Thoem, 1993).

The present work aimed to study the bacterial causes of enteritis of cows and buffaloes and pathological changes associated with these infections, in two abattoirs at Ismailia Governorate.

MATERIALS & METHODS

A total of 1500 samples had been collected from 336 cattle and 164 buffaloes aged between 3 months to 2 years old, slaughtered in Ismailia and El- Tal El- Keber

abattoirs. All samples were collected from apparently healthy animals. Tissue specimens from liver, small intestine and mesenteric lymph nodes were taken aseptically and subjected to bacteriological examinations. Proper samples from these organs were kept in 10% neutral formalin for histopathological examinations. Isolation of bacterial pathogens was carried out according to Quinn *et al.* (1994) using Selanite F. broth (Difco), Rappaport Vassiliadies broth (Oxoid), Brolac agar (Merck) and XLD agar, SS agar, Brilliant green agar Mac-Conkey's agar, Nutrient agar, EMB agar media (Oxoid). Biochemical characterization of isolates were performed according to Cruickshank *et al.* (1975). Isolates proved to be *Salmonella* were serologically typed according to Kauffmann-White Scheme (Kauffmann, 1972) using *Salmonella* antisera (Wellcome reagents limited). Phage typing was done by Dr. Raili in Department of Bacteriology in National Veterinary and Food Research Institute in Finland. Tissue specimens for histopathological examinations were taken and immediately fixed in 10% buffered neutral formalin solution, then, paraffin sections of 5 microns thickness were prepared and stained with hematoxylin and eosin according to Bancroft *et al.* (1990).

RESULTS

Bacteriological Examinations

The total numbers of positive animals for enteric bacterial isolates from cows were 244 out of 336 (66.6%), and 97 out of 164 (59.2%) from buffaloes with a total percentage of 68.2% (Table 1). The predominant isolates was found to be *E. coli* with a percentage of 28% in cows and 25% in buffaloes. The incidence of salmonella, Enterobacter and proteus was 14.3, 10.1and 9.8 percentage in cow samples, while, it was 10.4, 4.9 and 6.1 percentage in buffalo samples.

Serological identification of 95 *Salmonella* strains isolated from 65 positive animals (48 cows and 17 buffaloes) are presented in Table 2. The strains were belonging to two serovars, *S. enteritidis* and *S. cerro.*

Salmonella enteritidis phage type 4 infection was detected in cows and buffaloes.

Pathological Examinations

Enterobacter, Citrobacter, Proteus, Klebsiella and *Edwardsiella* caused no pathological changes in the intestine, mesenteric lymph nodes and liver of positive cases. The most prominent lesions in these organs were seen in *Escherichia coli, Salmonella*

Table 1. Number of positive animals for bacterial isolation.

Isolated organism	Cows (No. 336)		Buffaloes (No. 164)		Total (500)	
	No.	%	No.	%	No.	%
E. coli	94	28	41	25.0	135	27.0
Salmonella	48	14.3	17	10.4	65	13.0
Enterobacter	34	10.1	8	4.9	42	8.4
Prodteus	33	9.8	10	6.1	43	8.6
Klebsiella	11	3.3	10	6.1	21	4.2
Citrobacter	16	4.7	5	3.1	21	4.2
Yersenia	8	2.8	2	1.2	10	2.0
Edwardsiella	-	-	4	2.4	4	0.8
Total	244	66.6	97	59.2	341	68.2

Table 2. Serotyping of the *Salmonella* strains Isolated from 336 cows and 164 buffaloes.

	Antigenic Structure			Cow		Buffalo	
	Somatic	Flagellar (H) Antigens					
Salmonella serovars	(O) group	phase I	phase II	No.	%	No.	%
S. enteritidis	1, 9, 12	G,m	1, 7	47	13.9	17	10.4
S. cerro	6, 14, 18	Z_4, Z_{23}	1, 5	25	7.5	6	3.7
Total				72	21.4	23	14.00

spp. and *Yersinia* infections.

In *E. coli* infection, the intestine showed no prominent gross lesions except few cases which showed congestion of the mucosa covered with slimy exudate. The mesenteric lymph nodes were enlarged and showed thickening of their capsule. The liver in some cases was enlarged in size and showed thickening of their capsule. The liver in other cases was enlarged in size and pale in colour. Microscopically, the small intestine showed desquamation of the epithelial cell lining of the intestinal villi with leukocytic infiltration mainly neutrophils and lymphocytes in the lamina propria and submucosa (Fig. 1). Some epithelial cells lining the intestinal villi showed vacuolation of the cytoplasm (Fig. 2). Degeneration and necrosis of the cell lining the intestinal glands were observed (Fig. 3). Depletion of lymphoid follicles of the Peyer's patches could be seen. Hyaline degeneration of the smooth muscles was detected in the tunica muscularis together with congestion of the blood vessels. The intestinal lymph nodes showed degeneration and depletion of lymphocytes of the lymph follicles (Fig. 4). Hyperplasia of the endothelial lining of blood vessels and thickening in their wall, perivascular oedema and leukocytic infiltration were also seen. The liver showed thickening and hyalinization of fibrous tissue of hepatic capsule. Hepatic cells showed vacuolation and focal necrosis (Fig. 5). Perivascuolar oedema and leukocytic infiltration were observed in the portal area (Fig 6). The hepatic blood vessels were congested.

In *Salmonella spp.* infection, the small intestine in some cases showed congestion, and the intestinal contents were watery and mixed with mucus. Mesenteric lymph nodes were enlarged and congested. Liver showed tiny white area on its surface, while, in few cases it was enlarged. Microscopically, the intestine showed desquamation of the epithelial cells lining the intestinal villi. Hyperplasia and metaplasia of the epithelial cells lining of the intestinal villi and vacuolation of the epithelial lining of intestinal glands were seen. The blood vessels in lamina propria and submucosa were congested and leukocytic cells infiltration, mainly neutrophils and lymphocytes, were seen in lamina propria and submucosa (Fig. 7). Round cells infiltration and oedema among the degenerated and necrosed intestinal gland in the submucosa were seen. Hyperplasia of the endothelial cells of the serosal blood vessels were seen, and also thrombus was noticed in some blood vessels. The intestinal lymph nodes showed focal degeneration and depletion of lymphocytes. There were necrotic cells scattered in the lymphoid follicles with oedema and haemorrhages. In some cases, activation and hyperplasia of the lymphoid follicle and hyperplasia of reticular cells were detected. The liver showed vacuolation of the hepatic cells, focal necrosis, as well as, increased number of Kupffer's cells (Fig. 8). The destroyed hepatic cells were replaced by focal macrophages, lymphocytes

and oedematous fluid. Focal aggregation of inflammatory round cells, haemorrhagic areas and coagulative necrosis were observed (Fig. 9). Moreover, the portal area showed fibrous tissue proliferation, oedema and few leukocytic infiltration with congestion of the portal blood vessels and newly formed bile ductule (Fig.10).

In *Yersinia* infection the liver and small intestine showed no gross lesions. Microscopically, the intestine showed desquamation of the epithelial cell lining intestinal villi. The lamina propria and submucosa were infiltrated with neutrophils and lymphocytes. Congestion of the blood vessels was seen. The mesenteric lymph nodes showed degenerative changes and coagulative necrotic areas surrounded with neutrophils and macrophages. In some follicles of the lymph nodes, there was focal eosinophilic homogenous round necrotic areas (Fig. 11). Liver showed necrotic foci as well as congestion of the portal blood vessels.

DISCUSSION

The present investigation revealed that the incidence of *E. coli* was 27%, *Salmonella* 13%, *Enterobacter* 8.4%, and *Prodteus* 8.6%. These results are in a partial agreement with Ali *et al.* (1996), Taha (1999) and Abd-Alla *et al.* (2000). The authors reported that the prevalent microorganisms in calves suffering from enteritis were *E. coli, Prodteus, Salmonella* and *Enterobacter.*

The incidence of *Salmonella* infection in cows and buffaloes was 14.2% and 10.3%, respectively which presented to two serovars, *S. enteritidis* and *S. cerro. Salmonella enteritidis* was previously isolated from an outbreak of salmonellosis in cows by Pafifi *et al.* (1992) in Italy, and Labib (1998) in Egypt, while, from buffalo, *Salmonella enteritidis* was recently recorded by Zaki (2000) with an incidence of 21.43%. The available literature on salmonellosis caused by *S. cerro* in buffaloes was found to be scarce. Few recorded serovars of *S. cerro* in cows were found. Labib (1998) isolated *S. cerro* in Egypt and also Wells *et al.*, (1999) recovered *S. cerro* from dairy cows in the U.S.

The pathological lesions due to enteric organisms were nearly similar with some differences due to nature of the causative agent and the degree of its severity.

The present studies revealed that *E. coli* infection in cows and buffaloes induced in the small intestine several lesions as desquamation of the epithelial cells lining of some villi as well as degeneration and necrosis of the epithelial cells lining the intestinal glands. These changes are in agreement with Jubb *et al.* (1993) and Taha (1999).

The degeneration and necrosis of the intestinal glands, and depletion of the lymphoid follicles of the Peyer,s patches could be attributed to cytotoxin produced by *E. coli* (Gyles and Thoem, 1993). The lymphoid tissue is a part of the immune system, so, destruction of the lymphoid tissue lowers the resistance of the animals.

El-hamamy *et al.* (1999) reported that the liver in enteric calves revealed vacuolar degeneration together with congestion of the hepatic blood vessels. These results are in a partial agreement with results of this study, where, thickening and hyalinization of the fibrous tissue of hepatic capsule were detected. This picture could be attributed to the toxin produced by *E. coli*. Moreover, the mesenteric lymph node showed degeneration and depletion of the lymphocytes of the lymphoid follicles. The enlargement of the mesenteric lymph nodes was due to infiltration of inflammatory cells (Jones and Hunt 1983).

The *Salmonella enteritidis* and *Salmonella cerro* caused in cows and buffaloes hyperaemic intestinal mucosa and watery yellowish intestinal contents, which were mixed with mucus. These results are in a complete agreement with Jones and Hunt (1983). On the other hand, Jubb *et al.* (1993) reported the presence of petechial haemorrhages on tunica serosa besides haemorrhagic and fibrinous exudate on the intestinal mucosa. Jubb *et al.* (1993), El-Hamamy *et al.* (1999) and Taha (1999) described desquamated epithelial lining of the intestinal villi, mucinous degeneration of the lining epithelial cells and infiltration with neutrophils and lymphocytes in the lamina propria and submucosa. Gyles and Thoem (1993) recorded that the enteritis due to Salmonellosis resulted in shortening of villi, degeneration and abnormal extrusion of enterocytes, increase emptying of goblet cells and a neutrophilic reaction in the lamina propria accompained by transepithelial migration of neutrophils to lumen. There were degeneration and necrosis in the epithelial cell lining of the intestinal glands which were attributed to cytotoxin produced by *Salmonella*. (Gyles and Thoem, 1993). Jubb *et al.* (1993) mentioned that the submucosal oedema and necrosis of the epithelial cell lining of the intestinal gland could be a result to ischemia due to micro vascular thrombosis. Thrombosis and congested blood vessels were seen in the submucosa and serosa. These results are in harmony with Gyles and thoem, (1993), and Taha (1999). Jubb *et al.* (1993) recorded that the vascular degeneration and thrombosis of the mucosal vessels are common features of *Salmonella enteritidis*. Vacuolar degeneration, focal necrosis of hepatic cells, and activation of Kupffer's cells were the picture of salmonellosis in the liver recorded by Jones and Hunt, (1983) and Jubb *et al.* (1993). The activation and hyperplasia of Kupffer's cells is an aid for phagocytosis and defense against the infection. The results of focal aggregation of inflammatory cells and hoemorrhagic area in

addition to fibrous tissue proliferation, oedema and leukocytic infiltration with congestion of blood vessels, go in harmony with Jones and Hunt (1983) and Gyles and Thoem (1993). Scattered necrotic cells were seen in the mesenteric lymph nodes with oedema and haemorrhages. These results are completely in conformance with Jones and Hunt (1983). Hyperplasia occurring in lymph nodes could be regarded as a mean for increase humoral antibodies produced by B cells and lymphocytes.

In the present studies, *Yersinia* infection showed mild lesion in the intestine. Jubb *et al.* (1993) reported that the microscopic lesion may not be detected in the intestinal mucosa of some clinically affected animals from which *Yersinia* was isolated. Liver showed necrotic foci and congestion of blood vessels; such results are in agreement with Jubb *et al.* (1993). They recorded that there may be mild fibrinous, cholecystitis and necrotic foci scattered in the liver. The Picture of yersiniosis in the mesenteric lymph nodes were similar to that recorded by Jones and Hunt (1983) and Jubb *et al.* (1993). Gyles and thoem (1993) mentioned that, in both intestinal tissue and lymph nodes, focal necrosis was observed in affected organs. The inflammatory response is less than would be excepted given the degree of tissue necrosis, and it has been suggested that the organisms were able to suppress the cell mediated immunity.

Finally, it can be concluded that the important enteric organisms among slaughtered cows and buffaloes in Ismailia gavernorate were *E. coli* and *Salmonella spp*. The percentage of *Salmonella enteritidis* and *Salmonella cerro* isolated from these animals cannot be neglected because salmonellosis is one of most important food borne disease.

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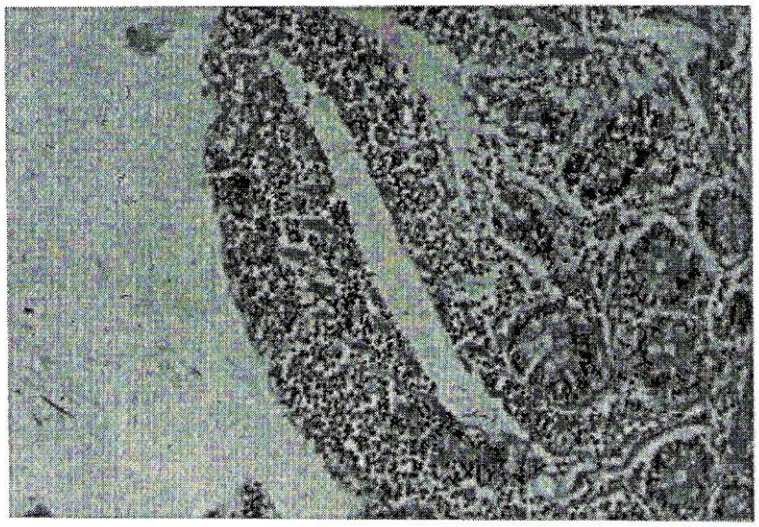

Fig 1. Intestine of a cow showing desqumation of the epithelial cells lining intestinal villi and leukocytic infiltration (H&E x.200)

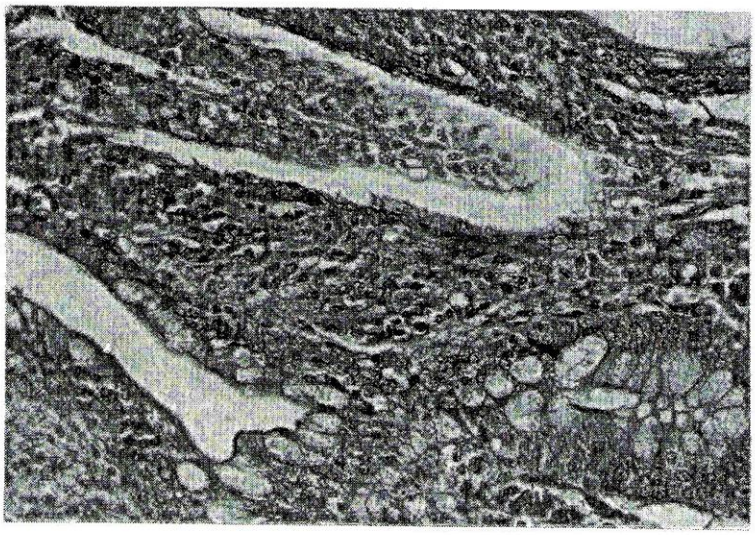

Fig 2. Intestine of a cow showing necrosis of the intestinal villi and vacuolation of the cytoplasm (H& E x.400)

Fig 3. Small Intestine of a cow showing necrosis of the intestinal glands to gather with leukocytic infiltration. (H& E x200)

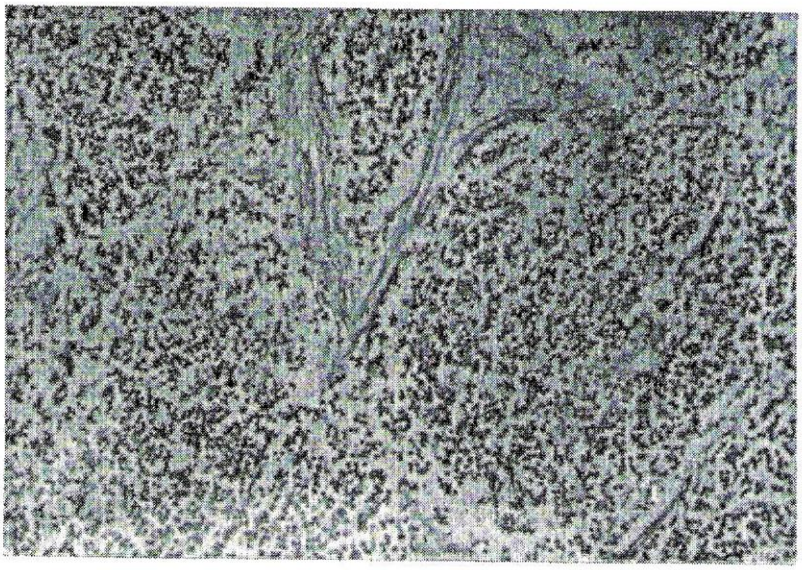

Fig 4. Mesenteric lymph node showing degeneration and depletion of the lymphocytes of the lymph follicles (H& E x.200)

Fig 5. Liver of a cow showing thickening of fibrous tissue of the hepatic capsule and vacuolation of the hepatic cells. (H&E x.400)

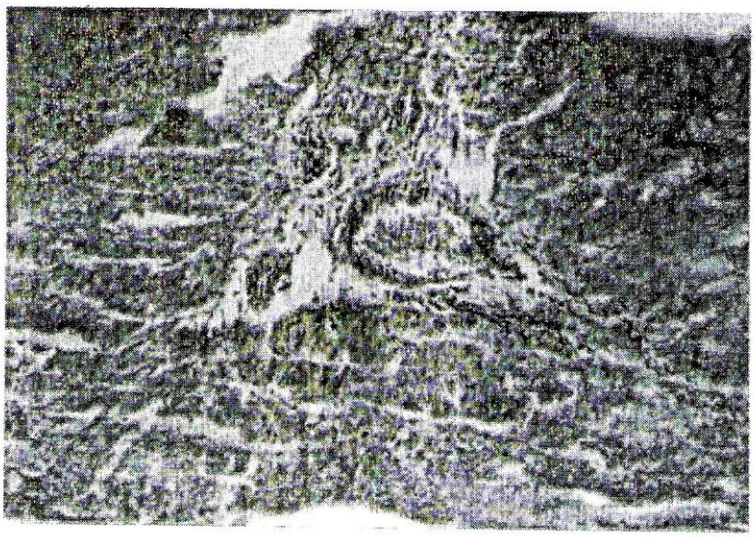

Fig 6. Liver of a cow showing perivascular oedema and leukocytic infiltration in portal area (H& E x.200)

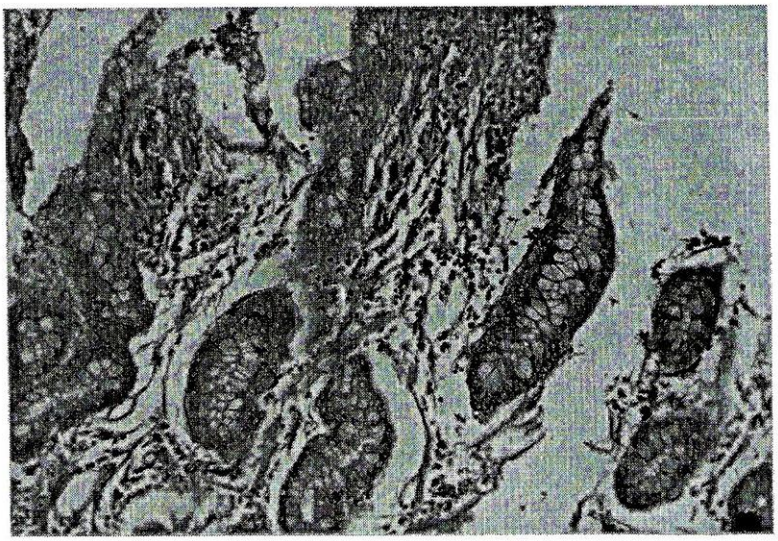

Fig. 7. small intestine of cow showing hyperpalasia of the epithelial cells of the intestinal villi, vacuolation of the epithellal cells lining intestinal gland and leukocytic infiltration.(H&E x 200)

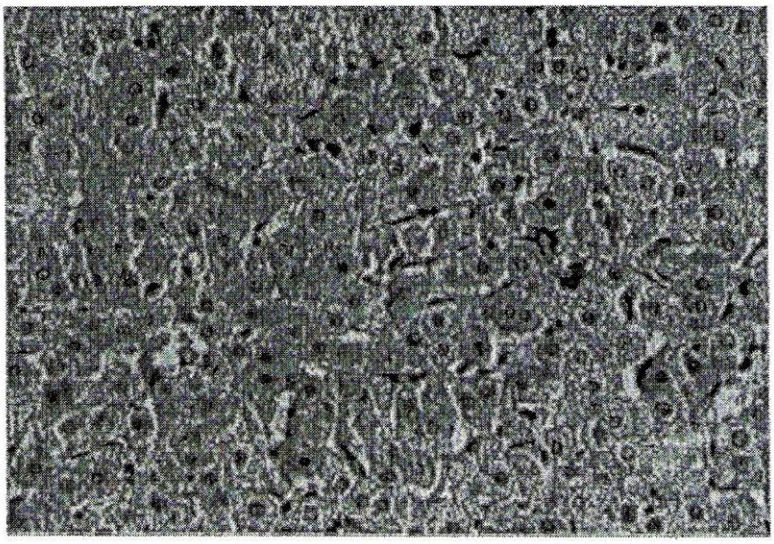

Fig. 8. Liver of a cow showing activation of kupffer cells necrosis of some hepatic cells with leukocytic infiltration (H& E x 400)

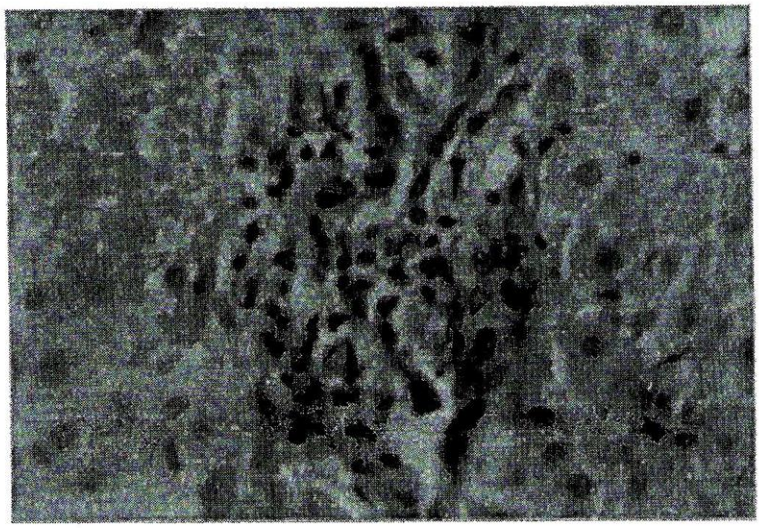

Fig 9. Liver of a cow showing focal aggregation of inflammatory cells, haemorrhagic area and coagulative necrosis of hepatic cells (H& E x 650)

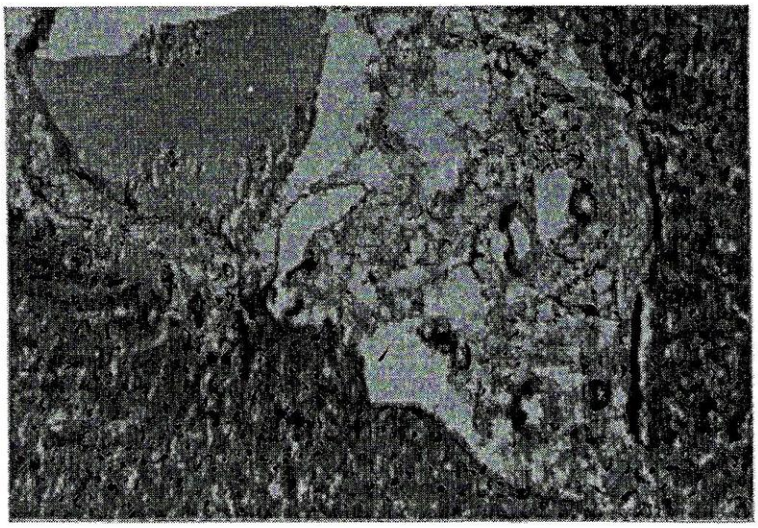

Fig. 10. Liver of a cow showing oedema in the portal area, fibrous tissue proliferation, leukocytic infiltration and congestion of portal blood vessels and newly formed bile ductules.(H&E x.200)

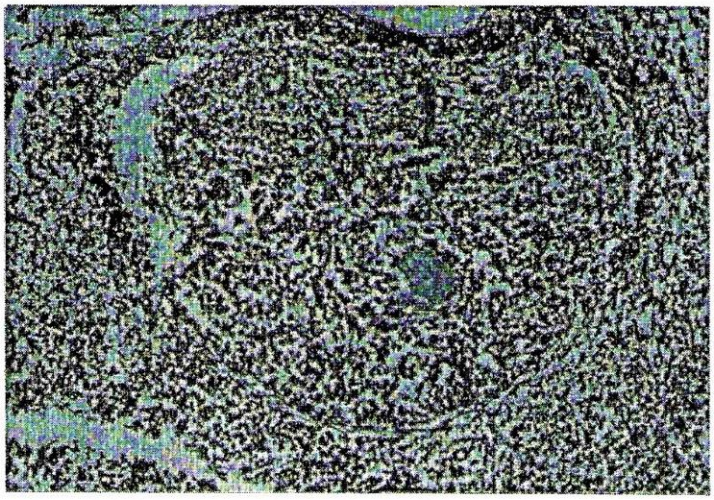

Fig. 11. Mesenteric lymph nodes of a cow showing focal eosinophilic homogeneus round necrotic area in the ceter of some lymph fallicles (H&E x .200).

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دراسات بكتريولوجية وباثولوجية عن الميكروبات المعوية مع إشارة خاصة للسالمونيلا فى الأبقار والجاموس المذبوحة بمحافظة الأسماعيلية

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تم إجراء الفحوص البكتريولوجية والباثولوجية على عدد ٥٠٠ حيوان (٣٣٦ رأساً من الأبقار، ١٦٤ رأساً من الجاموس) مذبوحة فى مجازر بمحافظة الإسماعيلية. تم أخذ عدد ١٥٠٠ عينة من تلك الحيوانات تمثلت فى الأمعاء الدقيقة ، الغدد الليمفاوية الملحقة بها والكبد. أظهر الفحص البكتريولوجى لتلك العينات أن متوسط نسب عزل الميكروبات المعوية فى الأبقار وصلت إلى٦٦,٦٪ وفى الجاموس ٥٩,١٪. تم تصنيف تلك الميكروبات إلى الإشريشيا القولونى والسالمونيلا والأنتيروبكتريا والبروتيس والكلبسييلا والستروباكتر اليرسينيا وأدوارسيلا. كانت نسب تلك المعزولات مختلفة فى البقر عنها فى الجاموس. تم تصنيف ميكروبات السالمونيلا المعزولة إلى سالمونيلا انتريديتس وسالمونيلا سيرو بنسب ١٣,٩٨٪ ، ٧,٤٧٪ فى الأبقار بينما كانت فى الجاموس ١٠,٤ ، ٣,٦٥٪ على التوالى.

لم تؤدِّ معظم تلك المعزولات إلى تغيرات باثولوجية واضحة فى الأعضاء التى خضعت للفحص. ولكن أدت الإصابة بميكروب الإيشريشيا القولونى والسالمونيلا واليرسينيا إلى حدوث تغيرات باثولوجية فى الأمعاء والغدد الليمفاوية المعوية والكبد والتى تفاوتت مظاهرها وحدّتها تبعاً لنوع الإصابة.