Some Studies On Bacteriological Causes Of Diarrhea In Rabbits

Azhar M Hassan, Neven A El Nisr and Eman, M A El.Naser
Dept. of Bacteriology, Dept. of Pathology and Dept of
Biochemistry and Nutritional Deficiency Diseases
Animal Health Research Institute, Assiut Lab.

ABSTRACT

Twenty–eight rabbits aged from 1-3 months of both sex were obtained from rabbits farm in Assiut governorate, 20 of them were suffered from diarrhea, and the others were apparently clinically healthy where 8 of them were chosen to be the control group.

Feacal samples were obtained directly from the rectum of each rabbit under aseptic conditions in sterile container. Bacteriological tests for the detection of the causative agents of diarrhea were done. The most important results of feacal examination were as follows: The incidence of pathogenic *E. coli* was 45%, the incidence of non–pathogenic *E. coli* was 30 %, the incidence of *Clostridium piliform* was 5%., the incidence of *Stapholoccus Spp* was 20%.

The gross picture was congestion of both liver and intestine which was filled with gasses and fluids. The liver was dark red in color. Microscopically the intestine showed atrophied villi, degenerated epithelium with edema, congestion and leukocytic infiltration in the lamina propria in case of E. coli and Clost. Piliform infection . In case of *S. aurous* infection, the intestine showed severe metaplasia of the intestinal epithelium to goblet cell in addition to neutrophil cell infiltration in the lamina propria. The liver showed severe necrotizing lesion with congestion in its vasculature.

There was a significant increase in the levels of erythrocyte counts (RBCs), packed cell volume (PCV), hemoglobin (Hb), total leukocyte counts (TWBCs), neutrophils , lymphocytes and total protein (albumin and globulin). These changes were usually associated with cases of diarrhea and loss of body fluids.

INTRODUCTION

The high cost protein and rapid increasing population in developing countries, are the major constraints militating against the availability of the much need high quality protein food to low income earners .This situation calls for the production of fast maturing animals like rabbits with the utilization of cheap and locally available feedstuff in order to produce them at an affordable cost *(1)*.

Diarrhea is a major clinical finding due to malabsorpation in the intestinal tract. There are many causes of enteritis or malabsorption in farm animals. Enteropathogenes include bacteria and viruses very fames to cause enteritis *(2)*.

Among the most common causes of intestinal diseases of microbial origin in developing countries are E. coli, toxins of Staph aureus and Clostridium Sp. *(3)*.

The symptoms of digestive problems accompanied with diarrhea in rabbits are simple and constant. The first sign, which last 1 to 3 days are generally not noticed. It is a decrease in food intake by the rabbit. After the 5th day moderate diarrhea accompanied by skin dehydration. Rabbits were considered to have diarrhea when feces were semisolid and their perinea or hind legs were wet and soiled *(2)*.

Although there are many studies on hematology of various domestic animals, few data are available about hematological values of farm rabbits, in healthy and diseased conditions, confirming the fact that the rabbit reared for meat production has received little or no attention in the developing nations *(4)*.

This study aimed to investigate some of the bacteriological causes of diarrhea in rabbits, pathological picture of their intestine and liver as well as the hematological and biochemical changes of these rabbits.

MATERIALS AND METHODS

A. Animals

Twenty diarrheic rabbits aged from 1-3 months were used in this study.

B. Sampling

1. Faecal samples

A total of twenty faecal samples were collected from diarrheic and apparently healthy rabbits. Each sample was inoculated into nutrient broth followed by incubation. Plating was carried out on the selective media.

2.Blood serum samples

Blood samples were collected by puncturing the jugular vein and allowing free flow of blood into labeled sterile universal bottles *(5)*. Pooled sample from each animal was divided into two parts. The first part, about 10 ml was collected over labeled sterile universal bottles containing EDTA. This was used to determine the hematological components *(5, 6)*. The second part, about 10 ml was collected without anticoagulant. The obtained clear blood serum sample was used for determination of biochemical parameters using spectrophotometer *(7)*.

3.Bacteriological and histopathological samples

After slaughtering the diseased rabbits, samples for bacteriological examination were taken from the intestine. For histopathological examination, specimens were taken from intestine and liver and fixed in 10% neutral buffered formalin. Paraffin section of 4 µm thickness were prepared and stained with hematoxylin and eosin (H and E) stain for microscopical examination *(8)*.

C. Adopted methods

Bacteriological studies

Isolation and identification of the microorganism.

A sterile loop full from samples (faecal, and intestine) was inoculated into a nutrient broth, incubated overnight at 37°C for enrichment and enhancement of bacterial growth. Subcultures were streaked on nutrient agar, 5% sheep blood agar and McConey bile salt media. After incubation, suspected colonies were described for their appearance, haemolytic activity and morphological characters. Smears from the colonies were stained with Gram stain and examined microscopically then divided according to staining, reaction, and shape and cell arrangement.

The isolates were identified biochemically *(9)*.

D. Statistical analysis

Statistical analysis of the obtained data was done by means of computer statistical performance *(10)*. Means, standard error and significant of variance were statistically achieved.

RESULTS

Clinical findings in diarrheic rabbits

The majority of rabbits suffering from diarrhea were weak, lethargic and anorexic.

Bacteriological isolation

Bacteriological isolation revealed that 9 isolates (45%) of pathogenic *E coli*, 6 isolates (30%) of non pathogenic *E. coli*, 2 isolates (20%) of *Staphylococcus aureus* and one isolate (5%) of *Clostridium piliform* (Table 1). Mixed infections from pathogenic and non pathogenic *E coli* were also detected in some cases.

Pathological results

Grossly: The intestine and the liver were congested and the intestine was filled with gases and watery content (Fig. 1, 2). The cecum was filled with gases and little fluid.

Microscopically: The most characteristic lesions were noticed in rabbits infected with *E. coli*. The intestine showed villus atrophy with severe degeneration in the intestinal epithelium (Fig. 3). The lamina propria was infiltrated with leucocytes mainly neutrophil,

plasma cells and some lymphocytes (Fig. 4). The lamina propria of the intestine in the affected rabbits was edematous and congested .In case of C. piliform infection there was focal segmental necrosis of the mucosa of the cecum (Fig. 5). There were also submucosal edema , necrosis of the muscular layers with leucocytic infiltration mainly heterophil cell . In case of *S. aureus* infection there were mainly increase number of the goblet cells and epithelial degenerative and necrobiotic changes (Fig. 6). The lamina propria was hyperemic, edematous with leukocytic infiltration (Fig. 7). The main microscopic lesion in the liver was circumscribed area of coagulative necrosis which reached to caseation in some cases fig (8). The hepatic vasculature were severely congested and surrounded with inflammatory cells mainly heterophil cells, macrophage cells and cell debris (Fig. 9).

Hematological results: were shown in Tables (2, 3 and 4).

Table 1. Percentage of some bacterial agents causes diarrhea in rabbits.

Bacterial causes	Percentage %
Pathogenic E coli	45
Non path. E coli	30
Staphylococcus Spp	20
Clostridium pill form	5

Table 2. Mean values of RBCs, PCV, Hb, MCV, MCH, and MCHC in healthy and diarrheic rabbits

Clinical status	RBCs (T/L)	PCV %	HB g/L	MCV fl	MCH Pg	MCHC g/dl
Healthy control rabbits	4.73±0.17	29.04±0.58	9.08±0.13	59.3±1.1	19.1±0.9	31.3±0.3
Diarrheic rabbits	5.9±0.2**	31.1±0.7*	10.4±0.1**	63.2±3.1**	21.6±0.7*	33.7±0.6**

Each value represents the means ± standard errors, Significance compared to control: {*P< 0.05,**P< 0.01}

Table 3. Mean values of total and differential leucocytic count of healthy and diseased rabbits

Clinical status	TWBCs G/L	Neutrophil %	Lymphocyt %	Monocyt %	Basophil %	Eosonophil %
Healthy rabbit (control)	7.17±o.2	31.6±1.7	60.2±1.6	1.7±0.5	1.1±0.2	1.6±0.5
Diarrheic rabbits	9.28±o.3**	33.3±0.8**	64.1±1.9**	1.8±0.6	0.8±0.3	1.2±0.5

Each value represents the means ± standard errors, Significance compared to control: {*P< 0.05,**P< 0.01}

Table 4. Mean values of total protein albumin and globulin of healthy and diarrheic rabbits

Clinical status	Total protein g/dl	Albumin g/dl	Globulin g/dl
Healthy rabbit (control)	5.68±1.24	2.92±0.81	2.83±0.62
Diarrheic rabbits	6.80±0.86*	3.64±0.21*	3.20±0.19*

Each value represents the means ± standard errors, Significance compared to control: {*P< 0.05}

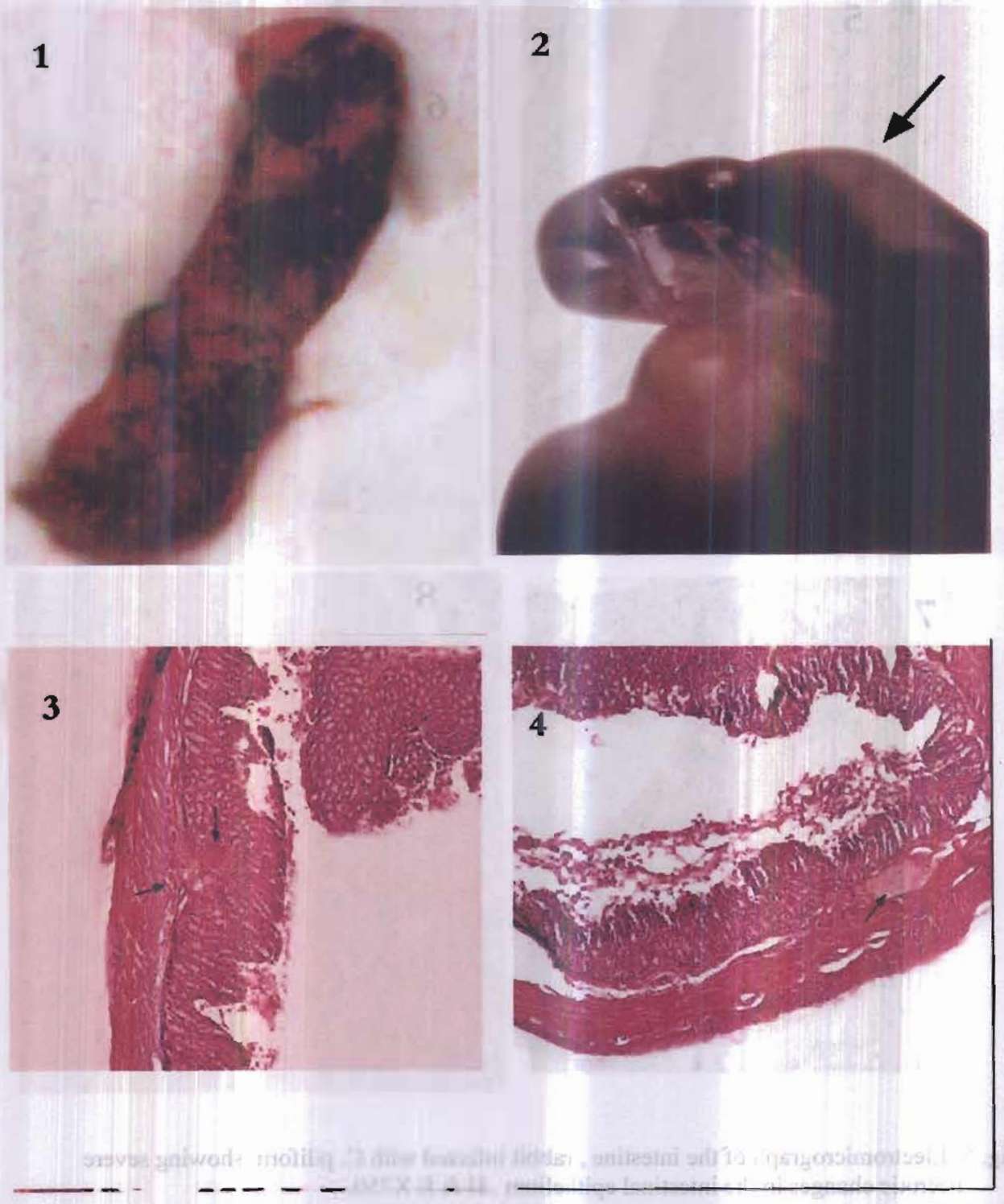
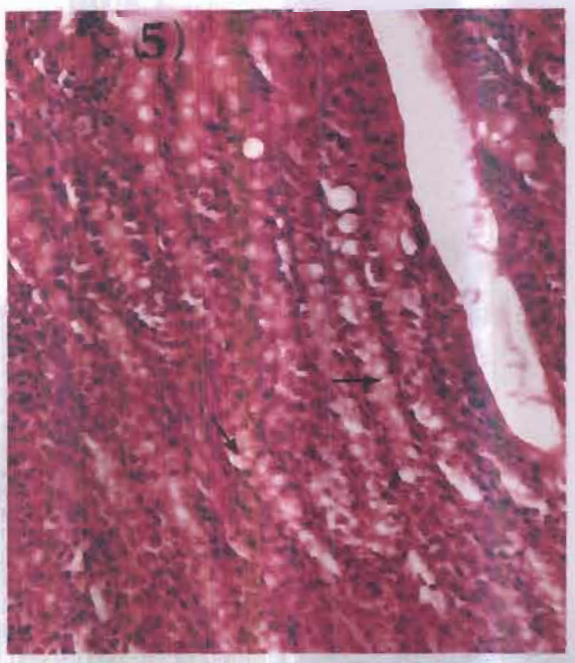
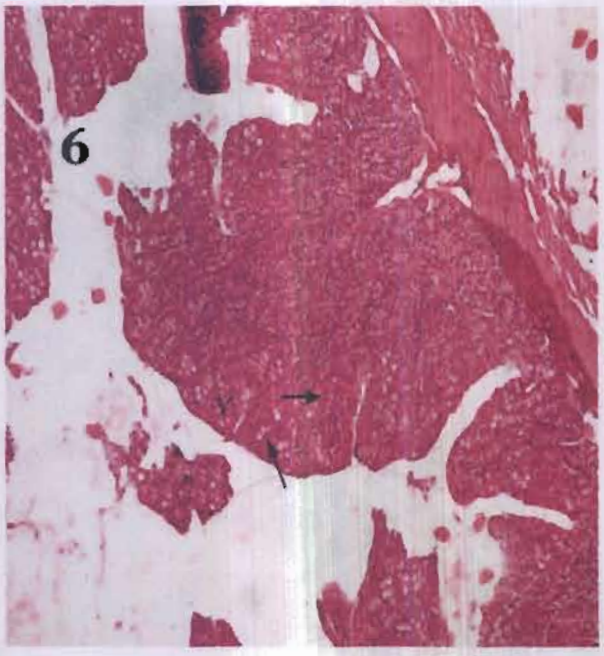


Fig.1. Intestine of rabbit showing enteritis with severe redness (congestion).
Fig.2. Intestine of rabbit showing enteritis with watery content and gases
Fig.3. Intestine of rabbit infected with E. coli showing cellular infiltration in the lamina propria (arrow) H & E X 300.
Fig. 4. Electromicrograph of the intestine ,rabbit infected with E. coli showing edema , congestion and hyperemia in the lamina propria (arrow) H& E X 300.





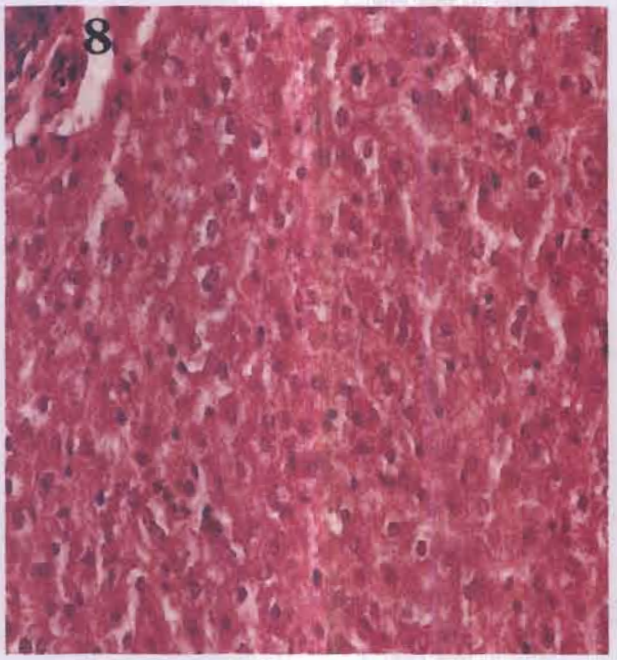


Fig. 5. Electromicrograph of the intestine , rabbit infected with C. piliform showing severe necrotic changes in the intestinal epithelium . H & E X750.

Fig. 6. Electromicrograph of the intestine , rabbit infected with S.aureus showing increase number of goblet cell (arrow). H&E X300.

Fig.7.Electromicrograph of the intestine ,rabbit infected with S.aureus showing increased number of the goblet cell, cellular infiltration and edema of the lamina propria. H&E X 750.

Fig. 8. Electromicrograph of the rabbits liver, showing severe necrosis in the hepatic cells. H&E X 750.

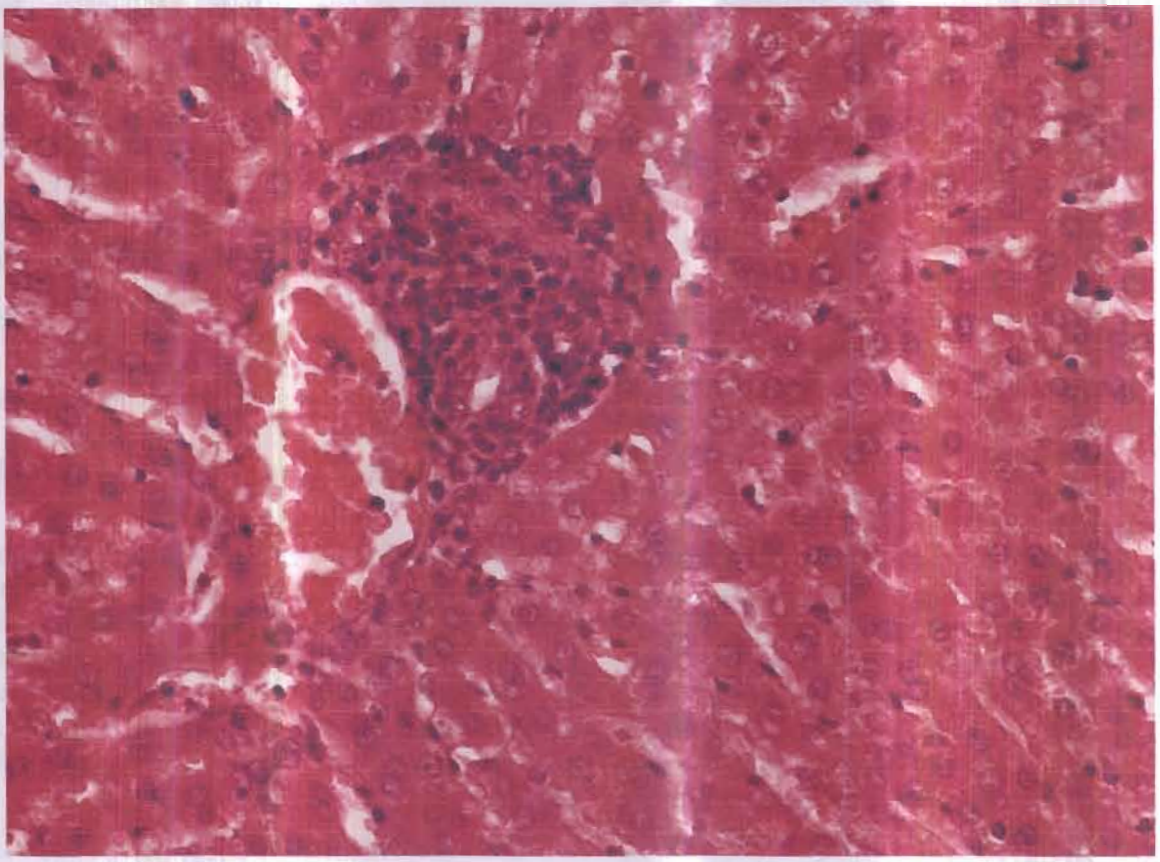


Fig. 9. Electromicrograph of the rabbits liver, showing congestion in the central vein which surrounded with heterophilic infiltration . H & E X750.

DISCUSSION

The bacterial populations in a rabbit's intestinal tract are considered the most delicately balanced of any in all herbivorous mammals. The growth and activity of normal (favorable) bacteria tend to keep potentially harmful bacteria in check ***(2)***. Overgrowth of harmful bacteria usually results in production of toxins that are rapidly absorbed into the rabbit's circulation, quickly causing illness and death. In addition to orally administered antibiotics can disturb the balance of bacteria. Rapid changes in the diet are most often implicated.

Escherichia coli considered one of the causes of rabbit diarrhea (Enteropathogenic strain of *E. coli*). The apparently healthy rabbits do not have *E. coli* of any strain associated with their gastro-intestinal tract. Diarrhea in rabbits infected with E . coli was severe and yellowish in color. Intestines are filled with water contents , rough coat as well as perineal area covered with greenish brown feacal materials. The incidence of pathogenic *E coli* was greatest under poor hygienic measures, poor husbandry and other stress factors. Other bacterial causes as *Staphylococcus sp.* and *Clostridium* accompanied by acute onset of profuse watery diarrhea, depression and dehydration. The obtained results are similar to those obtained in weaned rabbits ***(11)***.

Grossly: The intestine was congested and filled with water contents. Enterotoxigenic *E. coli* infection cause dilated, flaccid small intestine which filled with translucent fluid ***(12)***

Microscopically: The most characteristic lesions were noticed in cases infected with *E. coli*. where the intestine showed villus atrophy with severe degeneration in the intestinal epithelium and the lamina propria was

congested, edematous and infiltrated with leucocytes mainly neutrophil, plasma cells and some lymphocytes. The same picture was has been recorded previous studies ***(13,14)*** which explain villus atrophy in human , rabbits and lamb by the ability of *E. coli* to attach to the luminal aspect of enterocytes on villi throughout the small intestine resulting in exfoliation of the affected cells and mild to severe atrophy of the villi which infiltrated with neutrophil ***(15)***. Furthermore the villous atrophy occurs in the intestine was response to the heat stable enterotoxins B. which secreted from enterotoxigenic *E. coli* ***(16)***. The *E. coli* infection cause damage of the surface epithelium which followed by hyperemia, edema and variable neutrophilic infiltration in the lamina propria ***(3)***. In cases of clostridial infection the main lesion was severe necrosis in the intestinal epithelium. Clostridium spp. Produce enterotoxins and cause epithelial damage ***(3,17)***. In case of *S. aureus* infection the pathological lesions were mainly increase number of the goblet cells and epithelial degenerative and necrotic changes, the lamina propria was hyperemic edematous with leukocytic infiltration. *S. aureus* is one of the pathogens associated with infectious diarrhea, the ability of *S. aureus* to cause this lesions may be due to the presence of peptidoglycan (PGN) which is a cell wall component of S. aureus also has strong immune activity as its capability of modifying the functions of some immune cells ***(18)***. Furthermore oral antibiotic therapy may cause enteritis by alteration of the intestinal flora due to over growth of other bacteria as Staphylococci which are normally kept under restraint. The cases from which *S. aureus* isolated showed edema in the intestine, leukocytes in the lamina propria, and increased number of goblet cells with large amounts of mucin ***(19)***. The liver lesions (necrotic changes of the hepatic cells, the heterophilic infiltration and vascular congestion) are similar to those previously described ***(17)***.

In healthy rabbits, the mean values of erythrocytic counts (RBCs), Packed cell volume (PCV), Hemoglobin (Hb), mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean Corpuscular hemoglobin concentration (MCHC), total white blood cell counts (TWBCs) and differential leucocytic counts shown in Tables 2, 3. The obtained data were found to be within the range reported by many authors for clinically healthy rabbits ***(6,5)***.

The mean values of RBCs, PCV, Hb, MCV, MCH, MCHC, showed high significant increase in the diarrheic rabbits (Tables 2). Such elevations are usually associated with enteritis and attributed to excessive loss of body fluids, heamoconcentration and reduction of vascular space. These findings are in agreement with those previously recorded ***(20,4)***.

The mean values of total leucocytic counts, neutrophils, and lymphocytes showed high significant increase in the enteric rabbits, (Table, 3). These findings could be attributed to bacterial and virus infections and inflammatory lesions that act as stimuli for production of neutrophils and lymphocytes ***(15,21)***

The increased total proteins (Table 4) observed during this study may be due to excessive loss of water via watery stool in diarrhea. The increased serum globulins may also be due to secretion of immunoglobulins to defend the body as described in previous studies ***(7,22)***.

CONCLUSION

Our result proves that, there were two causes of bacterial enteritis caused by *E. coli* and *S. aureus*. Pathologically: the intestine in bacterial enteritis was characterized by atrophy of the intestinal villi and necrotic changes in the intestinal epithelium, the lamina propria showed inflammatory cell infiltration .Mainly neutrophil cells in case of bacterial enteritis. The main heamatological indices of diarrhea in rabbits were hemoconcentration (increase of RBCs, Hb, and PCV).An increase in the total white blood cell counts (TWBCs), neutrophils and lymphocytes. Some biochemical parameters like total protein, albumin and globulin also showed increase in the diarrheic rabbits. Hematological and biochemical parameters can yield information about the red

blood cell population, leukocyte and biochemical responses to pathogens.

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الملخص العربي

بعض الدراسات على المسببات البكتريولوجية للإسهال في الأرانب

ازهارمحمد حسن ، نيفين عبد الغني النسر، ايمان محمد عبدالناصر

أجريت هذه الدراسة علي الأرانب التي تتراوح اعمارها بين ١ الي ٣ شهور في إحدى مزارع أسيوط وكانت تعانى من الاسهال وحالات النزلة المعوية و قد وجد ان ٢٠ حالة منها مصابة بالاسهالات الشديدة وقد اختير عدد ٨ من الأرانب التي تبين من الفحص الاكلينيكي لهم انهم بصحة جيدة ولا توجد أعراض ظاهرية لأي امراض اخري و استخدمت كضابط للدراسة. اخذت العينات من الارانب السليمة ظاهريا والمصابة بالاسهال وقد تم تجميع عينات براز للفحص البكتريولوجى . وأخذت عينات مصل الدم للفحص الكامل لصورة الدم. و قد أثبتت النتائج. ان أسباب الالتهابات المعويه البكتيريه هى ميكروب القولوني المعدي والميكروب العنقودى الذهبى.

أوضحت النتائج:الآتى: ١.عزل البكتريا المعوية (الميكروب القولونى) بنسبة ٤٥% من الارانب المصابة بالاسهال. ٢. عزل البكتريا المعوية (الميكروب القولونى) الغير مرضي بنسبة ٣٠ % من الارانب المصابة بالاسهال. ٣. عزل المكيروب العنقودى بنسبة ٢٠% من الارانب المصابة بالاسهال. ٤.عزل ميكروب الكلستريديم بنسبة ٥% من الارانب المصابة بالاسهال.

وكانت الصفة التشريحية متشابهة لكل هذه المسببات وهى احتقان الأمعاء وامتلائها بالغازات والسوائل واما الفحص الهستوباثولوجى فقد بين ان الألتهاب المعوى الناتج عن الميكروب الذهبي العنقودي يتميز بزيادة كبيره في عدد الخلايا الكأسيه الموجوده بين الخلايا الطلائيه وكان هناك احتقان شديد فى الأوعيه الدمويه وانتشار الخلايا متعادلة الصبغه فى النسيج الضام للأمعاء

وبإجراء الفحوصات الدموية وجد ان هناك زيادة في قيم كل من عدد كرات الدم الحمراء (RBC) و حجم الترات المصمطة (PCV) بالاضافة الي الهيموجلبين (Hb) وايضا في عدد كرات الدم البيضاء (TWBCs), بخاصه الخلايا المتعادلة والخلايا الليمفاويه وكذلك زيادة فى البروتين الكلى والالبيومين والجلوبيلين فى مصل الدم وذلك لاضطرابات سوائل الجسم .الناتجة عن الاصابة بالاسهال و الالتهابات المعوية