SOME STUDIES ON ENTERITIS IN RABBITS

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(Manuscript received 12 Octobre 1998)

Abstract.

One hundred and fifty diseased and freshly dead rabbits were examind for mycotic and bacterial organisms. The examined rabbits showed diarrhoea and/or enteritis. The most recovered mycotic organisms were *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Mucor racemosus* and *Candida albicans*. The recoved bacteria were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris*. Serotyping of *Escherichia coli* isolates revealed 4 different servovars; O119, 0128, 0157 and 0178. Salmonella was not isolated from rabbits. Groups of 5 NewZealand White weaned rabbits of 30 days old were used to test the pathogenicity of isolated *Aspergillus fumigatus, Mucor racemosus, Geotrichium candidum, Candida albicans* and different serovars of *Escherichia coli*. Mixed infection of Aspergillus fumigatus, Candida albicans and Escherichia coli was also studied. Clinical and pathological changes were recorded. The main pathological changes were congestion, haemorrhages and necrosis in most examined organs, in addition to presence of periodic acid schiff (PAS) positive fungal spores in hepatic and intestinal tissues.

INTRODUCTION

During recent years, rabbits industry became well established in Egypt. The rabbit meat is used as a good source of animal protein, and some breeds are reared for fur production, as well as, for medical and biological purposes.

Enteritis remains one of the major problems facing rabbitries causing high mortalities which is of economic importance. This problem is of mutiple etiology. The mycotic enteritis associated with diarrhoea was previously reported in different animals (El-Badri, 1992 and Jensen *et al.*, 1994). *Candida* species and Aspergillus fumigatus were isolated from fatal gastrointestinal lesions in calves and proved lethal for rabbits, mice and guinea pigs (Barinov, 1968).

The disorders of the digestive tract of rabbits due to bacterial agent were recorded by many authors allover the world (Urosevic *et al.*, 1986, Abdel Gwad,

1988 and Harwood, 1989). Hegazy *et al.* (1992) isolated *Klebsilla* and *Arizona* from diseased or freshly dead rabbits with history of high mortality, diarrhoea and lesions of septicaemia

This study was planned to investigate the mycotic, as well as bacterial infections associated with enteritis in rabbits to find out the possible role of the isolates in causing diseases in experimentally infected rabbits and to clear the pathological changes associated with this infection.

MATERIALS AND METHODS

1. Samples

Liver, small and large intestine of 150 diseased and freshly dead rabbits with diarrhoea and/or enteritis were subjected to mycotic and bacterial examinations. The samples were collected from Kaluobia, Sharkia and Giza Provinces.

2. Mycotic examination

Direct microscopic examination

Scrapings from liver, small and large intestine of diseased and freshly dead rabbits were examined directly with microscope using 20% potassium hydroxide, carbol fuchsin and Gram's stain.

Cultural examination

Small fragments of the examined organs were immersed in 70% ethyl alcohol for 5 minutes, then, inoculated into Sabouraud's dextrose agar containing penicillin (20 I.U/ml) and streptomycin (40 mg/ml), and incubated at 37°C for 10 days. Suspected yeast and mould growth were subcultured onto Sabouraud agar slants in order to obtain pure cultures and are kept for futrther identificant. *Aspergillus* colonies were inoculated on Czapeck's agar (3%), Peicillium colonies were cultured on Czapeck and Malt extract agar and other colonies were cultured on Potato dextrose and Malt extract agar for mould species identification. Yeasts were identified by culturing on Rice agar, the presence of germ tubes in serum and biochemical reactions.

3. Bacteriological examinations

Cultural examination

Loopfuls from liver, small and large intestine were inoculated aseptically in to

Selenite-F broth (Difco) for 8-10 hours at 37°C, then, platted on Salmonella Shigella (Oxoid) agar at 37°C for 24 hours. The plates were examined for the presence of *Salmonella* suspected colonies.

Another loopfuls from the same orgas were identified morphologically and biochemically according to Cruickshank *et al.* (1975), Koneman *et al.* (1988) and Carter and Chegappa (1991).

Serological identification

Serological identification of suspected *Escherichia coli* strains was pointed out according to Edwards and Ewing (1972). Serotyping of the isolates was performed with slide agglutination test using *Escherichia coli* polyvalent and monovalent "O" atisera obtained from DENKA SEIKEN Co. LTD., Tokyo, Japan.

4. Experimental infection

Fifty apparetly healthy NewZealand White weaned rabbits of 30 days old were divided into 10 groups consisting of 5 each. The groups were used to test the pathogenicity of isolated *Aspergillus fumigatus, Mucor racemosus, Geotrichium candidum, Candida albicans* and different serovars of *Escherichia coli*. Mixed infection of *Aspergillus fumigatus, Candida albicans and Escherichi coli* was also made. Before infection, random samples were subjected to mycological and bacteriological examination which proved to be negative for infection. The last group was kept as control. Each rabbit was inoculated orally with 5-ml of spores suspension containing 2.6×10^9 spore/ml for each fungal species (Chihaya *et al.*, 1988). The rabbits received a suspension of 2×10^6 CFU of each *Escherichia coli* serovar (Peeters *et al.*, 1984).

Animals of all groups were kept under observation for up to 21 days with record of the clinical signs and mortalities. At the end of the experiment, survived rabbits were sacrificed. Recently dead, as well as sacrificed rabbits were subjected to post-mortem, mycological and bacteriological examinations for resisolation of inoculated organsims.

5. Pathological examination

A small specimen from different organs including liver, small and large intestine of freshly dead or sacrificed experimentall rabbits were immediately fixed in 10% formol saline for histopathological examination. The samples were then washed with distilled water, and then, routinely stained with Hematoxylene and Eosin and Periodic Acid Schiff (P.A.S.) (Clayden, 1971).

RESULTS

Clinically diseased rabbits were depressed, diarrhoeic, off food and had ruffled fur.

Grossly, there was congestion of internal organs. Liver showed enlargement, and some cases showed necrotic foci and/or distended gall bladder. Intestine showed catarrhal enteritis with fluid contents and gases. Some cases showed dark coaecal contents with or without haemorrhages of cecal mucosa. Lung congestion was reported in few cases.

The incidence of mycotic affection in examined rabbits is showed in Table 1. It is clear that, *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Mucor racemosus* and *Candida albicans* were isolated from liver, small and large intestine. The incidence of fungal infection in liver, small and large intestine was 4%, 14.67 and 14.67%.

Table 1. Incidence of mycotic affections in examined rabbits.

Isolates	Liver		Small intesine		Large intestine	
	No.	%	No.	%	No.	%
Aspergillus fumigatus	1	0.67	2	1.33	2	1.33
Aspergillus flavus	2	1.33	2	1.33	1	0.67
Aspergillus niger	1	0.67	2	1.33	2	1.33
Aspergillus glucacus	0	0	2	1.33	1	0.67
Aspergillus chevalieri	0	0	0	0	1	0.67
Cladosporium cladosporiodes	0	0	0	0	3	2
Mucor racemosus	1	0.67	2	1.33	2	1.33
Mucor species	0	0	4	2.67	1	0.67
Penicillium citrinum	0	0	2	1.33	0	0
Rhizopus species	0	0	0	0	2	1.33
Scopulariopsis brevicaulis	0	0	0	0	1	0.67
Tricoderma species	0	0	0	0	1	0.67
Candida albicans	1	0.67	2	1.33	2	1.33
Candida parpsillosis	0	0	2	1.33	1	0.67
Geotrichium candidum	0	0	2	1.33	2	1.33
Total	6	4	22	14.67	22	14.67

Incidenc of different bacteria isolated from examined cases appeared in Table 2. It is observed that, the incidence of total bacterial isolation in liver, small and large intestine was in percentage of 8.67%, 18% and 27.33%, respectively. The

recovered bacteria were *Eschrichia coli* (44), *Klebsiella pneumoniae* (18 isolates), *Pseudomonas aeruginosa* (11 isolates) and *Proteus vulgaris* (8 isolates).

The examined livers were positive for *Escherichia coli* and *Klebsiella pneumoniae* only in incidence of 7.33% and 1.33%, respectively, while, small and large intestine were positive for all recovered bacteria.

Serological identification of Escherichia coli isolates revealed that, out of 44 isolates, 21 (47.73%) were 0119, 14 (31.82%) were 0128, 6 (13.64%) were 0157 and 3 (4.55%) were 078.

Results of experimental infections of isolated fungi, *Escherichia coli*, serovars and mixed infection of both organisms were illustrated in Table 4. It is noted that the inoculated fungi were pathogenic and resulted in mortality of 20% except that *Aspergillus fumigatus* was 40%. Mortality rate of 60%, 40%, 20%, and 20% were reported in rabbits infected with *Escherichia coli* serovars 0119, 0128, 0157 and 078, respectively. Mixed infection of 0119, *Aspergillus fumigatus* and *Candida albicans* resulted in high mortality of 80%. The results of re-isolation of inoculated organisms from experimental rabbits were positive. The inoculated rabbits showed depression, dullness, off food and diarrhoea.

Postmortem examination of infected rabbits revealed liver congestion in case of Aspergillus fumigatus, *Mucor racemosus*, *Candida albicans* and *Escherichia coli*, with presence of small whitish foci in *Aspergillus fumigatus* and large pale areas with distention of gall bladder in *Candida albicans*. Congestion of mesentric vessels and haemorrhagic areas on hepatic surface were noticed in *Geotrichium candidum* infection. Catarrhal enteritis was recorded in *Mucor racemosus* and *Geotrichium candidum* infected rabbits. Intestine showed congested blood vessels with oedematous wall in *Aspergillus fumigatus* infection. Inflammatory intestial wall was noticed in *Escherichia coli* infection. *Candida albicans* and mixed infected cases showed pasty content and gases in intestinal lumen.

Microscopic findings of liver showed hepatic congestion with focal degenerative changes of hepatocytes in *Aspergillus fumigatus, Mucor racemosus* and *Candida albicans* infections. Severe congestion with focal areas of haemorrhages was observed in *Geotrichium candidum* and *Escherichia coli* infections, associated with hemosidriosis in the mixed infection. Focal mononuclear inflammatory cellular aggregation mostly lymphocytes was noticed in *Mucor racemosus* infection. In case of Candida *albicans* infection, focal areas of necrosis surrounded by mononuclear inflammatory cells and fibrous connective tissue proliferation with fungal masses at

periphary of necrosis were demonstrated (Fig. 1). The fungal masses were positive with PAS stain.

Examination of intestine revealed congestion and submucosal inflammatory oedema (Fig. 2), focal proliferation of lining epithelium and hyperplasia of goblet cells with presence of PAS positive fungal masses in subsmucosa of *Aspergillus fumigatus* in infected rabbits. Congestion of intestinal submucosal capillaries with monouclear cellular infiltration was seen in *Mucor racemosus* and *Geotrichium candidum* in infected cases. In addition, *Mucor racemosus* gave vacuolation of the glandular epithelium, while, focal hyperplasia of mucosal epithelium was noticed in *Geotrichium* cases. *Cadida albicans* infection produced congestion of blood vessels with proliferation of mucosal epithelium (Fig. 3). Oedema of submucosa infiltrated with inflammatory cells mostly lymphocytes and macrophages was also noticed. In *Escherichia* coli infection, focal desquamation of mucosal epithelium, hyperplasia of goblet cells with inflammatory cellular infiltration of lamina propria and submucosa mainly lymphocytes were noticed (Fig. 4). In mixed infection, congestion of blood vessels, desquamation of goblet cells proliferation with presence of PAS positive fugal spores were observed (Fig. 5).

DISCUSSION

Enterities and diarrhoea among rabbits cause high morbidity and mortality rates. Little is known regarding the aetiologic role of fungi in this problem. In the present study, *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Mucor racemosus* and *Candida albicans* were isolated from intestine and liver of diseased and dead rabbits. Nearly similar results were recorded by Refai *et al.* (1974) and Refai *et al.* (1990) who succeeded in the isolation of *Mucor, Aspergillus niger, Aspergillus fumigatus*, and *Aspergillus flavus* from the lungs, liver and hearts of hens, turkeys, ducks and rabbits. *Aspergillus gluacus* was isolated from small and large intestine of diarrhoeic rabbits, while, *Scopulariopsis brevicaulis* and *Tricoderma* species were recovered from large intestine of diarrhoeic rabbits. Ainswarth and Austwick (1955) reported one outbreak of scour in steers which was thought to be due to the consumption of mouldy grass nuts which when examined were found to be covered with a heavy growth of *Aspergillus gluacus, Tricoderma koningii* and *Scopulariopsis brevicaulis.* It is of interest to record that these three fungi were also the most frequent isolates from faeces of affected animals. Also, *Penicillium citrinum, Mucor* spp., *Rhizopus* spp., *Cladosporium cladosporoides*, Candida parpsillosis and Geotrichium candidum were isolated from intestine of rabbits with enteritis. Nearly similar results were obtained by Abou-Gabal *et al.*

Table 2. Results of bacteriological examination of examined rabbits.

Type of examined organs	No.of Examined organs	Total No.of Bacterial isolates	%	Type of isolated bacteria							
				Escherichia coli		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Proteus vulgaris*	
				No.	%	No.	%	No.	%	No.	%
Liver	150	13	8.67	11	7.33	2	1.33	0	0	0	0
Small intestine	150	27	18	14	9.33	6	4	4	2.67	3	2
Large intestine	150	41	27.33	19	12.67	10	6.67	7	4.67	5	3.33

(1977), Ibrahim *et al.* (1983) and Shalaby and Helmy (1992) in diarrhoeic fowls and Watanabe *et al.* (1976) in diarrhoeic cattle and pigs. Moreover, Kharole *et al.* (1976) found that *Rhizopus* and *Candida* species isolated from diarrhoeic Ragheb,buffalo-calves were highly pathogenic to rabbit by I/V inoculation, while (1990) recorded that *Candida albicans* and *Mucor* species isolated from diarrhoeic calves were pathogenic to rabbits and mice. On the other hand, *Geotrichium candidum* infected the mucous membranes of the alimentary tract of animals (Carter and Chengappa, 1991).

In the present study, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were isolated from examined rabbits (Table 2). Our results are supported by Sadek and El-Agroudi (1963) who isolated *Proteus* organisms from 2 baby rabbits with severe diarrhoea. *Proteus vulgaris* was isolated from rectal swabs of apparently healthy rabbits (Miligy and Ghoneim, 1970). Ali (1983) revealed *Proteus* spp., *Klebsiella* spp. and *Pseudomonas aeruginosa* from rabbits with digestive diseases, and Abdel Gwad (1988) isolated *Klebsiella pneumoniae* from liver of 100 dead rabbits. Katoch *et al.* (1993) isolated *Proteus* spp. and *Klebsiella* spp. from rectal swabs of 35 rabbits with digestive disorders. Since no pathogenicity tests were done on these organisms reported in this work, their role as a pathogen in enteritis in rabbits cannot be stated. Further work is needed to prove their pathogenicity.

The examined livers were positive for *Escherichia coli* and *Klebsiella pneumoniae*, while, small and large intestine were positive for all recovered bacteria. This may be attributed to the presence or absence of bacteraemia, and /or differences between the capability of certain pathogens in attacking and effacing the intestinal epithelia.

Table 3. Serovars of *Escherichia coli* isolated from examined rabbits.

Isolated serovar of *Escherichia coli*	Liver	Small intestine	Large intestine	Total
0119	5	8	8	21
0128	4	4	6	14
0157	1	1	4	6
078	1	1	1	3
Total	11	14	19	44

Our isolation of 44 *Escherichia coli* serovars 0119, 0128, 0157 and 078 (Table 3) was in agreement with Loliger *et al.* (1969), Varga and Pesti (1982), Abdel Gwad (1988), Jakic (1989) and Hegazy *et al.* (1992). On the other hand, *Salmonella* was not isolated from diseased or dead rabbits in this world. This result goes hand to hand with that mentioned by Chandra and Ghosh (1992) who examined 123 faecal samples from diarrhoeic and apparently healthy rabbits and failed to isolate *Salmonella*. Harwood (1989) reported high mortality in rabbits in a commercial rabbitary and isolated *Salmonella typhimurium* from the alimentary and systemic sites from these rabbits. This variation in isolation of *Salmonella* may be due to hygienic measurements and management of the examined flocks.

Table 4. Results of oral infection of 30-day old rabbits with isolated fungi and *Escherichia coli*.

Group No.	Inoculted organism	No. of inoculated rabbits	No. of deaaths	Percenatge %
1	*Aspergillus fumigatus*	5	2	40
2	*Mucor racemosus*	5	1	20
3	*Candida albicans*	5	1	20
4	*Geotrichium candidum*	5	1	20
5	*Escherichia coli 0119*	5	3	60
6	*Escherichia coli O157*	5	2	40
7	*Escherichia coli O157*	5	1	20
8 9	*Escherichia coli O119+ Aspergillus fumigatus + Candida albicans*	5	1	80

Regarding the experimental infections of isolated fungi, *Escherichia coli* serovars and mixed infection of both organisms (Table 4), it was clear that, *Aspergillus fumigatus* was the most pathogenic inoculated species with mortality percentage (40%). Pure culture of the organisms was recovered from liver and intestine of dead rabbits. Idris *et al.* (1981) diagnosed *Aspergillus fumigatus* infection as the cause of sudden death in four out of 87 cattle fed on Aspergillus *fumigatus* contaminated sunflower cakes. *Mucor racemosus* and *Candida albicans* gave the same mortality (20%). The pathogenicity of *Mucor* species and *Candida albicans* was previously reported by Carter and Chengappa (1991) and Chihaya *et al.* (1991). Barinov (1971) diagnosed fatal mycotic gastroenteritis in young calves under one month of age due to *Aspergillus, Mucor* and *Candida infections. Geotrichium candidum* produced diarrhoea and clinical symptoms without death. Migaki *et al.* (1982) reported watery diarrhoea attributed to *Geotrichium candidum* in six adults gorillas.

Under condition of this study, mortality rate ranged from 20-60% was reported in rabbits infected with *Escherichia coli* serovars 0119, 0128, 0157 and 078. These results are nearly similar to findings of Ali (1983), Hegazy *et al.* (1992) and Saad (1994). Differences in pathogenicity within and in between serovars may be attributed to the fact that different strains within a given serovar vary in pathogenicity. Mixed infection of *Escherichia coli* 0119 and *Aspergillus fumigatus* and *Candida albicans* resulted in higher mortality (80%). This indicated that the combined effect of fungi and *Escherichia* coli resulted in high deaths when compared to the effect of either fungi or *Escherichia* coli alone.

Regarding the pathological changes in infected rabbits, the degenerative changes of hepatic tissue and enteritis by *Asprgillus fumigatus* were also supported by Hassan and Selim (1984) who reported that *Aspergillus fumigatus* was highly pathogenic to rabbits. In *Mucor racemosus* infection, catarrhal enteritis, congestion and inflammatory changes in liver agreed with Jensen *et al.* (1994) who reported acute necrohaemorrhagic lesions in *Mucor* gastrointestinal lesions. Focal hepatic haemorrhages with hepatocellular degeneration and vacuolation of mucosal epithelium and submucosal inflammatory edema were found in intestine of *Geotrichium* infected rabbits. This finding agrees with Sheey *et al.* (1976) who recorded a case of *Geotrichium* septicaemia. Concerning the experimental *Candida albicans* infection, necrosis of hepatic cells with presence of PAS fungal spores were observed. Intestine showed congestion, proliferation of epithelium and goblet cells with inflammatory cellular infiltration. This results were in agreement with Chihaya *et al.* (1991).

Experimental *Escherichia coli* infection was associated with hepatic congestion, haemorrhages and catarrhal enteritis. These findings were similar to those of Prescott (1987), Coussement *et al.* (1984), Urosevic *et al.* (1986), Percy *et al.* (1993) and Saad (1994) who showed congestion and enlargement of liver and catarrhal enteritis with an increase in the fluid of bowl contents with or without gas in the intestine of rabbits inoculated with *Escherichia coli.*

ACKNOWLEDGEMENT

We gratefully acknowledge Dr. Tantawi, A.A., Lecturer of Pathology, Fac. Vet. Med., Moshtohor, for his help throughout this study.

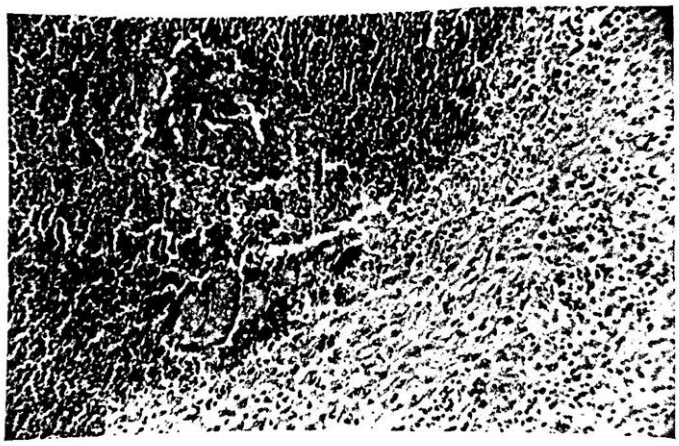

Fig. 1. Liver of rabbit orally infected with *Candida albicans* showing large central area of necrosis with presence of fungal masses at periphary of necrosis surrounded by thick connective tissue proliferation. H & E stain X 200.

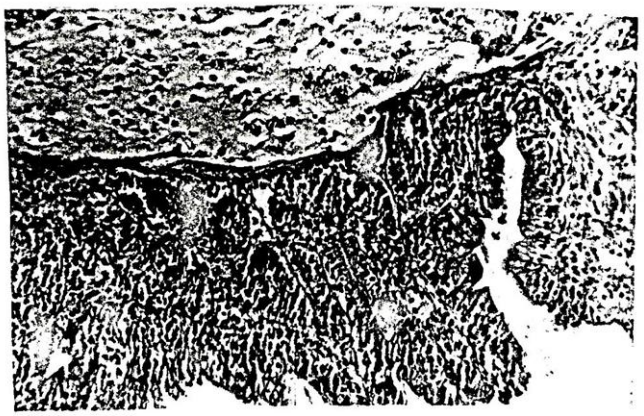

Fig. 2. Intestine of rabbit orally infected with *Aspergillus fumigatus* showing congested blood vessels and inflammatory oedema of submucosa. H & E stain X 200.

Fig. 3. Intestine of rabbit orally infected with *Candida albicans* showing proliferation of mucosal epithelium. H & E stain X 200.

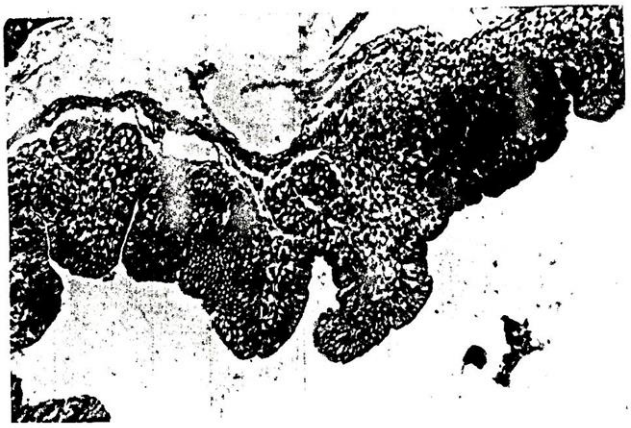

Fig. 4. Intestine of rabbit orally infected with *Escherichia coli* showing focal activation of goblet cells with inflammatory cellular infiltration in lamina propria mostly lymphocytes. H & E stain X 200.

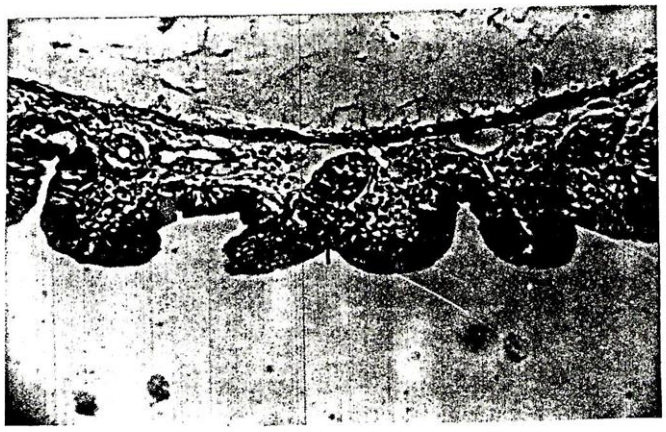

Fig. 5. Intestine of rabbit orally infected with *Aspergillus fumigatus Candida albicans* and *Escherichia coli* showing presence of PAS positive fungal spore. PAS stain X 100.

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دراسات علي الإلتهابات المعوية في الأرانب

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تم فحص ١٥٠ أرنبا مريضا ونافقا حديثا للفطريات والبكتريا. الأرانب التي فحصت كانت تعاني من اسهالات أو التهابات معوية. معظم الفطريات المعزولة كانت الأسبرجلس فيوميجاتس، الأسبرجلس فلافس، الأسبرجلس نيجر، الميوكر راسيموسس والكانديدا البيكانس، بينما كانت البكتريا المعزولة هي الميكروب القولوني، الكلبسيلا نيموني، السودوموناس ارجينوزا والبروتيس فالجاريس، أنتمت الأنواع السيرولوجية المعزولة للميكروب القولوني الي ٤ أنواع سيرولوجية مختلفة (0119, 0128, 0157 & 0178) كما لم يتم عزل ميكروب السالمونيلا من الأرانب. تم عمل عدوي تجريبيه لمجموعات من الأرانب النيوزيلاندي عمر ٣٠ يوما لأختبار مدي ضراوة الأسبرجلس فيوميجاتس، الميوكر راسيموسس، الجيوتريكم كانديدم، الكانديدا البيكانس والميكروب القولوني المعزولة من الأرانب المريضة. كما تم عمل عمل أصابة مشتركة من الأسبرجلس فيوميجاتس، الكانديدا البيكانس والميكروب القولوني. وقد سجلت الأعراض الأكلينيكية والباثولوجية. كانت معظم التغيرات الباثولوجية هي أحتقان، نزيف وتتكرز في معظم الأعضاء المفحوصة بالأضافة الي وجود حويصلات القطريات في انسجة الكبد والأمعاء بصبغة (PAS).