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MUCOID ENTEROPATHY AS FIELD PROBLEM IN RABBIT FARMS IN DAKAHLIA GOVERNORATE

(With 4 Tables and 10 Figures)

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ميوكويد انتيروباثي كمشكلة حقلية في مزارع الأرانب في محافظة الدقهلية

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فى هذة الدراسة تم فحص عدد ١٢٠ ارنب تتراوح اعمارهم من اسبوع الى ١٠ اسابيع (٩٠ نافق حديثا ، ٢٠مريض، ١٠سليم ظاهريا) تم جمعها من مزارع مختلفة بمحافظة الدقهلية اثناء فصلى الخريف والشتاء (٢٠١٠-٢٠١١) وقد تبين من الفحص الظاهرى للارانب المريضة وجود ضعف عام واسهال مخاطى شديد وانخفاض فى درجة الحرارة ونسبة نفوق عالية تتراوح ما بين ٥ – ١٥% في المزارع المصابة وباجراء الصفة التشريحية تبين وجود انسداد بالقولون مع وجود كميات كبيرة من المخاط والغازات بالامعاء واحتقان بالاعضاء الداخلية. وبالفحص البكتيريولوجى للعينات وجد ان ٩٢ حالة (٧و٧٦%) ايجابية للعزل البكتيرى وان ٤٠ حالة (٤٣,٥%) عدوى فردية و٥٢ حالة (٥٦,٥ %) عدوى مختلطة وكان الميكروب القولونى من اهم المعزولات فى ٩٨ حالة (٦٨,١%) يلية الميكروب العنقودى الذهبى فى ٢٧ حالة (١٨,٧%) ثم ميكروب الكلوستريديا برفيرنجيس نوع أ فى ١٩ حالة (١٣,٢%). تم تصنيف الميكروب القولونى العصوى سيرولوجيا الى٥ عترات و٢٥ معزولة غير مصنفة. كما تم عمل اختبار الحساسية للميكروبات المعزولة حيث كانت معظم المعزولات البكتيرية حساسة لكل من الانروفلوكساسين والجنتاميسين بينما ميكروب الكلوستريديا برفيرنجيس نوع أ كان حساس لكل من الامبيسيللين والبنسيللين. الفحص المجهرى اوضح زيادة عدد الخلايا الطلائية للخملات المبطنة للامعاء وسقوط قمم الخملات مع وجود العديد من الخلايا الطلائية مكونة المخاط بداخل الامعاء كما لوحظ تنكرز وتحول الخلايا المبطنة للخملات والغدد المعوية الى خلايا طلائية مع وجود خلايا التهابية واسطوانات ومخاط بداخل الامعاء. ومما تقدم يجب اتباع وسائل الامان الحيوى والصحى بمزارع الارانب مع استخدام العليقة المتزنة والمضادات الحيوية الفعالة لتقليل الخسائر الاقتصادية الناجمة عن مرض الميوكويد انتيروباثي في الأرانب الصغيرة.

SUMMARY

This work was carried out on 120 rabbits (90 dead, 20 diseased and 10 apparently healthy) of different breeds during autumn and winter seasons (2010-2011), their ages varied from one to 10 weeks. The suspected cases were collected from different private farms in Dakahlia Governorate. The

clinical sings were abdominal distention, subnormal temperature, polydepsia, anaroxia, severe watery mucoid diarrhea and high mortality rate from 5-15% among infected farms. The necropsid rabbits revealed partial or complete obstruction of colon with large plug of clear gelatinous mucous. Moreover, the other portions of gastrointestinal tracts were filled with fluid or pasty content together with gas. Liquid or dried fecal matter and mixed or coated with mucous were seen in the intestine of some examined rabbits. Bacteriological examination of the samples showed 92 (76.7%) positive bacterial isolation of which 40 (43.5%) single isolates and 52 (56.5%) mixed isolates. *E. coli* was isolated at incidence percentage 98(68.1%), *Staph. aureus* 27 (18.7%) and *Closteridium perfringens type A* 19 (13.2%). *E. coli* isolates were identified serologically into 16 (O119), 12 (O124), 15 (O125), 12 (O126) and 18 (O128) together with 25 untypable isolates. In vitro sensitivity pattern of isolated strains proved that Enrofloxacin and Gentamycin were the most effective drugs for most isolates while the *Clostridium perfringens type A* isolates were sensitive to Penicillin and Ampicillin. Histopathologically, the lesions were mainly confined to small and large intestines as a target organs. Goblet cell metaplasia among the epithelium of the intestinal villi and glands were noticed, accompanied with desquamated epithelial shreeds and mucous were observed inside the intestinal lumen. In severe cases necrosis and desquamation of intestinal tips were seen in some rabbits. Inflammatory cells with mucous and epithelial cast could be observed in the intestinal lumen in complicated cases. It could be concluded that good hygienic conditions, balanced nutrition and effective antibiotics play an important role in prevention and control of mucoid enteropathy in young rabbits.

Key words: *Rabbits, mucoid enteropathy, antibiogram, entevitis.*

INTRODUCTION

Rabbit production is a growing industry in Egypt, which proved economically profitable, digestive disorders are the main cause of morbidity and mortality in fattening rabbits and is responsible for important economic losses among rabbit farms (Okerman, 1987; Hatab and Moustafa, 2007).

At the end of 1996 a new clinical syndrome has emerged in French intensive enclosed rabbit farms. This disease was first named enterocolitis and is now called epizootic rabbit enteropathy (LeGall *et al.,* 1998; Licois, 1998; Licois and Caudert, 1999). This disease has spread from France to other countries and is now endemic on the European countries. The

mortality rate at onset of the epizooty were (30-80%) (Licois *et al.,* 1998; Marlier and Vindevogel, 1998; Licois *et al.,* 2005). This disease was observed in Egypt among rabbit farms causing severe losses in last years.

Mucoid enteropathy (MEP) is a distinct diarrheal disease of rabbit characterized by minimal inflammation, hyper secretion and accumulation of mucus in small and large intestines. It may occur due to fed of the young rabbits on low fiber and high starch diets results in high concentration of starch in the cecum and colon which facilitate invasion of intestinal mucosa by different intestinal flora. Bacterial toxins produced during the fermentation process may damage the mucosal surface (Wilber, 1999). Predisposing factors included stress, enzyme deficiency, change in acidity of the cecum, dietary changes, infectious agents and enterotoxins were responsible for occurrence of (MEP) (Brown 2002). Enteropathogenic *E. coli* was the most predominant causative agent of mucoid enteropathy in young rabbits sometimes accompanied with other bacteria as *Clostridium spp*., *Staph. aureus* and *Klehsiella spp*. (Dean *et al.,* 1993; Licois, 2004; Lavazza *et al.,* 2008; Hassan *et al.,* 2009).

This study was designed to investigate mucoid enteropathy as field problem in rabbit farms with special emphasis on its clinical signs, pathological lesions, isolation and identification of the bacterial agents and antibiotic sensitivity pattern for the bacterial isolates.

MATERIALS and METHODS

Samples:

This study was applied on 120 rabbits (90 freshly dead, 20 diseased and 10 apparently healthy) of different breeds during autumn and winter seasons (2010-2011). Their ages ranged from one to 10 weeks old and were obtained from different private farms in Dakahlia Governorate. Clinical signs and post mortem lesions suggestive presence of MEP. Samples from liver, spleen, kidney, small and large intestine, heart blood and the content of the caecum of freshly dead and diseased rabbits and cloacal swabs were taken from apparently healthy rabbits.

Bacteriological examination:

All samples were inoculated into nutrient broth, cooked meat broth and selenite F-broth and incubated at 37^{o}C for 18 – 24 hours followed by subculturing on nutrient agar, 5% sheep blood agar, MacConky's agar and Xylose lysine deoxycholate agar plates (Oxoid) and incubated aerobically and anaerobically at 37^{o}C for 18-24 hours. The growing colonies on various plates 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. The isolates were identified biochemically (1ndole, Nitrate reduction, Citrate utilization, H2S production, Gelatin liquefaction, Urease test, Nagleŕs test, Sugar fermentation and Coagulase test) according to Cruickshank *et al.* (1982); Finegold and Martin (1982) and Koneman *et al.* (1994).

Serological identification of *E. coli*:

Serological identification of purified *E..coli* strains using available agglutinating coli test sera (Behring Werk, AG Marburg) according to manufacturer's Instruction. Labn, Germany).

In vitro antibiotic sensitivity test:

The disc diffusion technique was performed on the isolated bacteria using Muller Hinton agar media (Oxoid). The chemotherapeutic disks were Enrofloxacin, Gentamycin, Erythromycin, Oxytetracycline, Ampicillin, Penicillin, Amoxycillin and Trimethprim Sulphamethoxazole. The degree of sensitivity was interpreted according to Koneman *et al.* (1994); Quinn *et al.* (1994); Oxoid Manual (1998).

Pathological examination:

Postmortem examination had been done to the freshly dead and diseased rabbits.Specimens from different segments of small and large intestine, were collected and fixed in 10% neutral buffered formalin. Paraffin sections of 5 microns thickness were prepared and stained with H & E according to Bancroft *et al.* (1996).

RESULTS

Bacteriological study:

Clinical signs:

The main clinical signs of diseased rabbits were high mortality (5-15%) deaths among different farms, ruffled fur, depression, off food, abdominal distention and mucoid diarrhea.

Postmortem lesion:

Affected rabbits showed complete obstruction of colon with large plug of clear gelatinous mucous. In severe cases, mucous cord impact the lumen of large intestine mainly cecum (Fig.1). Liquid or dried fecal matter mixed or covered with mucous were seen in the intestines of some other rabbits. The results of bacteriological examination were recorded in tables 1,2,3 and 4.

Table 1: Bacteriological examination of rabbits

Source of samples	Total No. of samples	Positive samples		Single isolates		Mixed* isolates		Total No. of isolates
		No.	%	No.	%	No.	%	
Apparently healthy rabbits	10	4	40.0	4	100.0	0	0.0	4
Diseased rabbits	20	16	80.0	6	37.5	10	62.5	26
Dead rabbits	90	72	80.0	30	41.7	42	58.3	114
Total	120	92	76.7	40	43.5	52	56.5	144

*Mixed infection with two microorganisms.

Table 2: Incidence of bacteria isolated from examined rabbits

Bacterial isolates	Apparently healthy rabbits		Diseased rabbits		Dead rabbits		Total	
	No.	%*	No.	%*	No.	%*	No.	%**
E. coli	3	3.3	16	17.4	79	85.9	98	68.1
Staph.aureus	1	1.1	7	7.6	19	20.7	27	18.7
Cl.perfringens type A.	0	0.0	3	3.3	16	17.4	19	13.2
Total	4		26		114		144	100.0

***** The percentage was calculated according to number of positive samples (92)**.**
** The percentage was calculated according to number of total bacterial isolates(144)

Table 3: Serotypes of *E. coli* isolated from examined rabbits

Serotypes	O_{126}	O_{119}	O_{124}	O_{125}	O_{128}	Untypable
Number	12	16	12	15	18	25

Table 4: Antibiotic sensitivity test for the bacteria isolated from examined samples of rabbits

Isolated bacteria / Antibiotic disc	*E.coli*	*Staph. aureus*	*Clostridium perfringens type A.*
Enrofloxacin $5u_g$	+ + +	+ + +	++
Gentamycin 10ug	+ + +	+ + +	R
Oxytetracycline $30u_g$	+ +	+ +	+ +
Amoxycillin $25u_g$	R	R	+ + +
Ampicillin 10ug	R	R	+ + +
Penicillin $15u_g$	R	R	+ + +
Erythromycin $15u_g$	R	+ +	+ +
Trimethoprim-Sulphamethoxazol 1.25 – 23.75ug	+ +	+ +	R

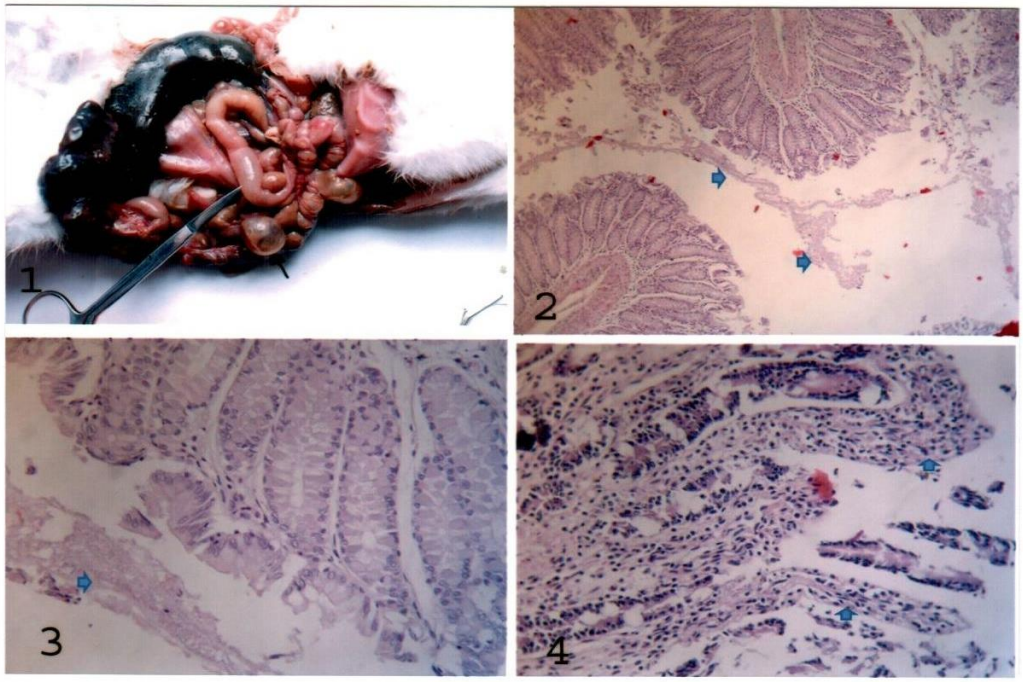

Fig. 1: Intestine of rabbits (MEP) showing presence of muocus (pointer) and gas (arrow) in the intestinal lumen beside impacted dark cacum.

Fig. 2: Large intestine of rabbits (MEP, *E. coli*) showing little muocus inside the intestinal lumen (arrow) H&E×300.

Fig. 3: The high power of the previous figure to show muocus and desquamated epithelial shreeds inside the intestinal lumen H & E × 1200

Fig. 4: Small intestine of rabbits (MEP, *E. coli*) showing severe necrosis and desquamotion of the intestinal tips (arrow) H & E × 1200

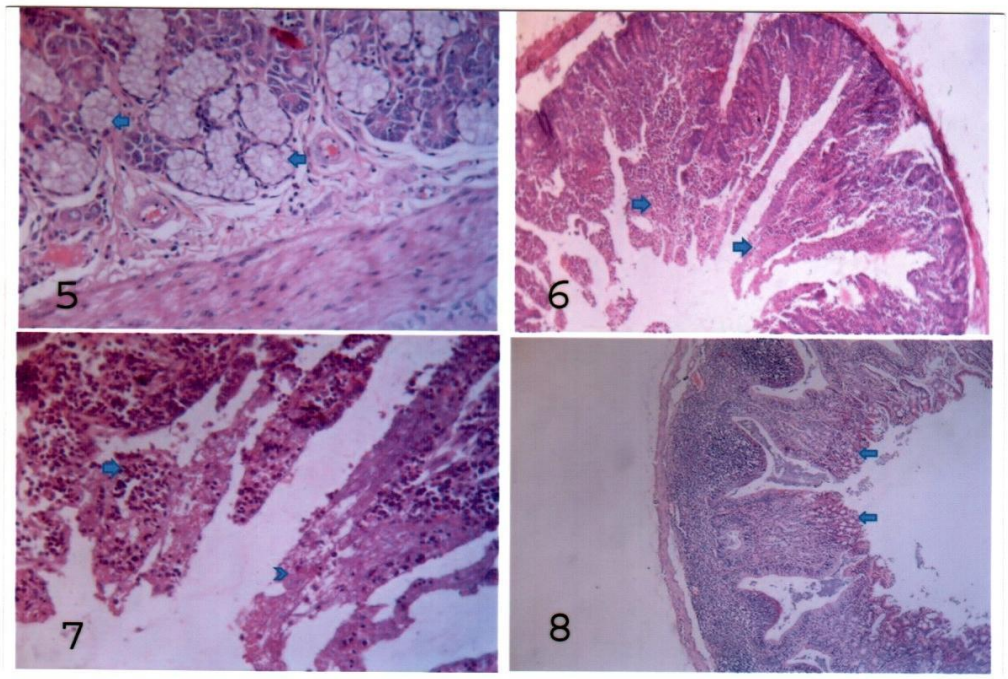

Fig. 5: Small intestine of rabbits (MEP, *E. coli*) showing metaplasia of the submucosal intestinal gland to goblet cell H & E × 1200.

Fig. 6: Small intestine of rabbits (MEP, *Clostridium perfringens type A*) showing mild infilammatory cells, muocus epithelial cast inside the intestinal lumen (arrow) H & E × 300.

Fig. 7: Small intestine of rabbits (MEP, *Staph. aureus*) suffered from necrosis and severe infiltration of inflammatory cells in the lamina propria H & E × 1200.

Fig. 8: Small intestine of rabbits (MEP, Staph. aureus) showing little muocus and numorous goblet cells on the surface epithelium H & E × 30

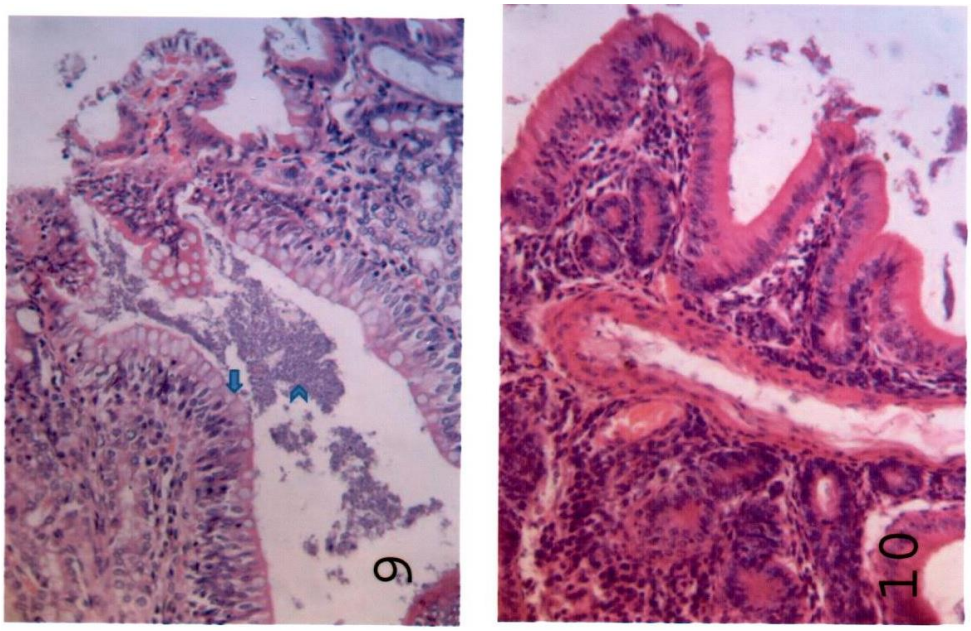

Fig. 9: Small intestine of rabbits (Negative bacterial infection) showing basophllic muocus, numerous goblet cells between the intestinal villi H & E × 1200.

Fig. 10: Apparently normal intestinal coat after treatment H & E × 1200

DISCUSSIONS

Enteritis is considered the major cause of disease in commercial rabbit industry. Diet, stress and management factors also acknowledged affecting the incidence and spread of enteric disease. Infectious agents known to play a role include parasites, enteropathogenic *E. coli* (EPEC) *Staphylococcus aureus* and *Clostridium spp*. (Percy *et al.,* 1993**;** Lavazza *et al.,* 2008). The bacterial infections are major health concern of rabbits production, one of the major causes of mortality in rabbits was gastroenteritis (Rai *et al.,* 1985). It was found that 120 cases of examined rabbits 92 (76.7%) revealed bacterial infection from which 40 (43.5%) yielded a single pure isolate and 52 (56.5%) yielded a mixed bacterial isolates (Table 1). On the other hand Hassan *et al.* (2008) recorded that the bacterial infection in newly born rabbits suffered from diarrhea was in single pure isolate 49 (50.52%) and mixed isolates 48 (49.48%). The clinical signs were depression, off food, emaciation, watery mucoid

diarrhea and death. The post mortem lesions in this study revealed congestion and edematous of the intestine with watery mucoid content beside congestion in all internal organs. The forementioned results are inagreement with those repored by El. Attar (1985); Rashed (2000); Hassan *et al.* (2008).

Bacteriological examination of the samples revealed that isolated bacterial pathogens were *E. coli* 98 (68.1%), *Staphylococcus aureus* 27 (18.7%) and *Clostridium perfringens type A.* 19 (13.2%). Lower incidence were recorded by Hassan *et al.* (2009) who isolated *E. coli* (45.0%), *Staph. spp.*(20.0%) and *Clostridium spp.*(5.0%), from rabbits affected with enteritis. On the other hand Hatab and Moustafa (2007) and Hassan *et al.* (2008) isolated *E.coli, Pasteurella multocida, salmonella spp., Staph aureus, Strept. pyogen, Klebsiella pneumoniae and Pseudomonus auregenosa* from diarrheic newly born rabbits. *Escherichia coli* is a Gram-negative, lactose fermenting, indole positive, facultative anaerobe of the human and animal intestinal flora. The organism typically colonizes the infant gastrointestinal tract within hours after birth, (Brasar and Hill, 1974). *E. coli* can adhere to the mucus overlying the intestine and destroy the microvilli of intestinal enterocytes. Enterotoxigenic E.coli (ETEC) also adheres to the enterocyte surface and cause disease by producing either heat labile toxin or heat stable toxin (Trenton, 1989). From our results in Table (2) *E. coli* was the most frequent isolates 98 (68.1%) which considered the main cause of rabbit mucoid enteropathy and mortality in young rabbits. The obtained results nearly similar to those obtained by (Peeters *et al.*, 1984; Percy *et al.*, 1993; Harcourt and Nigel 2002; Edrees *et al.*, 2008). They concluded that *E.coli* infection is the most and primary causative agent in most outbreaks of enteritis and diarrhea in young rabbits. From Table (3) it's clear that 73 out of 98 identified *E.coli* isolates could be identified serologically into 12 (O_{126}), 16 (O_{119}), 12(O_{124}), 15 (O_{125}) 18 (O_{128}) and 25 untypable, most *E. coli* serotypes isolated from apparently healthy, diseased and dead newly born rabbits were inagreements with those recorded by Ibrahim (1985); Percy *et al.* (1993); Hatab and Moustafa (2007); Hassan *et al.* (2008). *Staphylococcosis* in rabbits is caused by *Staph.aureus* which was isolated 27 (18.7%) from 1 (1.1%), 7(7.6%) and 19 (20.7%) of examined apparently healthy, diseased and dead rabbits respectively Table (2). Abdel-Gwad *et al.* (2004) isolated *Staph. aureus* from diseased rabbits with an incidence of (22.4%). Also Hatab and Moustafa (2007), Hassan *et al.* (2008) and Hassan *et al.* (2009) they isolated *Staph. aureus* from rabbits suffered from diarrhea with percent of 16.7%, 19.6% and 20.0% respectively.

Clostridium perfringens type A were isolated 19(13.2%) from diseased rabbits 3 (3.3%) and 16(17.4%)dead rabbits. Higher percentage was recorded by Maghawry and Nasr (2009) who isolated *Closteridium spp*. (43.8%) from diarrhoeic rabbits. Some authors isolated *Clostridium spp*. from diarrhoeic young rabbits (Peeters *et al.,* 1984; Dewree *et al.,* 2007; Edrees *et al.,* 2008; Lavazza *et al.,* 2008; Hassan *et al.,* 2009).

In Vitro sensitivity testing of isolates revealed that the most isolates of *E. coli* and *Staph aureus* were highly sensitive to Enrofloxacin and Gentamycin (Table 4). Similar results were reported by Abd El Rahman *et al.* (2005); Hatab and Abdel-latif (2006); Hassan *et al.* (2008). Concerning *Clostridium perfringens type A* it was highly sensitive to Amoxycillin, Ampicillin, Penicillin and Enrofloxacin. Nearly similar result was recorded by Maghawry and Nasr (2009) who found that *Clostridium perfringens* which isolated from diarrheic rabbit were sensitive to Penicillin and Enrofloxacin.

Pathological studies:

This study declared the Mucoid enteropathy, resulted in high economic losses among the infected rabbits. These losses due to mortalities (5-15%) and reduction in meat gain due to enteric lesions.

Moreover, this syndrome characterized by clinical signs which were depressions, off food, emaciations, watery mucoid diarrhea and ruffled fur. The fore mentions signs were inagreement with Dean *et al.* (1993); Licois (2004); Ashraf (2004). These signs occurred due to the hyper-secretions and accumulations of mucous in small and large intestine due to feeding on diet of low fiber and high starch. These facilitate the invasion of intestinal mucosa by different intestinal flora (*E. coli*, *Clostridium spp*. and *Staph*.). The bacterial toxins which resulted due to fermentation causing damage the mucosal surface (Wilber, 1999).

The main gross lesions in our work were obstruction of colon with large blood of cleared gelatinous mucous. In severe cases mucous cord impact the lumen of large intestine mainly cecum were common (Fig. 1). Moreover, the other segments of small and large intestine were filled with fluid or pasty content and gases. Similar results were recorded by El-Attar (1985); Coudert *et al.* (1997); Licois *et al.* (1998); Rashed (2000); Hassan *et al.* (2008).

Microscopically, *E. coli* was isolated in the most examined rabbits (98) at incidence percentage of (68.1%). These rabbits showed desquamation of the epithelial lining covering the large intestine with mucous content inside its lumen (Figs. 2 & 3). The small intestine showing severe necrosis and desquamation of the intestinal tips (Fig. 4). Metaplasia of the submucosal intestinal glands to goblet cells with thickening of the

muscular coat were also seen (Fig. 5). These results were agreement with those mentioned by Manning *et al.* (1994); Rashed (2000); Brown (2002); Hassan *et al.* (2008) who reported the some results in rabbits suffered from mucoid enteropathy with *E. coli* infection. *Clostridium perfringens type A* were isolated from 19 (13.2%). The small intestine of these rabbits showed mild inflammatory cells, mucous and epithelial casts inside the intestinal lumen (Fig. 6).

Staph. aureus infection 27 (18.7%) in all examined rabbits, the intestinal mucosa suffered from necrosis and sever inflammatory cell infiltration in the lamina propria (Fig.7). Moreover, little mucous and numerous goblet cells on the surface epithelium were seen (Fig.8). Basophillic mucous and numerous goblet cells between the intestinal villi were observed. Negative bacterial infection cases with mucoid enteropathy (Fig. 9). After using the sensitive drugs, the intestine noticed apparently normal without any lesions (Fig.10). Our results were nearly agreement with those obtained by many researchers (Wilber, 1999; Ashraf, 2004; Licois, 2004; Dewree *et al.*, 2007; Hassan *et al.*, 2008).

In our opinion mucoid enteropathy mostly occurred in the young rabbits post weaning due to certain predisposing stress factors such as sudden change in diet, enzyme deficiencies due to immaturity of gastrointestinal tract, feeding on diet of high carbohydrates and low fiber contents. All these factors resulting in changes in the acidity of the intestines with disruption of intestinal microflora. This microflora produced non inflammatory substances which stimulated the secretion of huge amount of mucous from the intestinal epithelium. This hyper secretion of the mucous could be considered as a compensatory mechanism to the hyperacidity of intestine (Check, 1987; Lelkes and Chang, 1987; Brown, 2002).

These investigations throw some lights on mucoid enteropathy syndrome in young rabbits and declared the bacterial pathogens and pathological changes of the intestine. So the good hygienic conditions, balanced nutrition and effective antimicrobial agents play an important role in the prevention and control of mucoid enteropathy in young rabbits.

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