Recent Status of *Clostridial* Enteritis Affecting Early Weaned Rabbits in Egypt

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Abstract: A surveillance study for diagnosis of ***Clostridial*** enteritis affecting early weaned rabbits was carried out on eight Egyptian governorates. Diagnosis based on history, clinical examination, palpation, post-mortem lesions, histopathological examination, as well as isolation of different ***Clostridial*** species (spp.) causing ***Clostridial*** enteritis. Two samples representing rectal swabs, liver and intestine were collected from each examined rabbits. A total of 718 samples expressing 329 surveyed rabbits (95 apparently healthy, 204 clinically affected and 30 freshly dead ones). Equal number (19) of feed and water samples were collected from each surveyed farm. All the samples were subjected for ***Clostridial*** isolation and spp. identification after cultural and biochemical characterization. Tissue samples from liver and intestine of freshly dead rabbits were subjected for histopathological examination. Results revealed that, the most prevalent observed signs were severe diarrhea, bloat accompanied with variable mortalities. Post-mortem lesions were severe enteritis and typhlitis with different degrees of necrosis and hemorrhages associated with gaseous contents. Both kidneys and livers showed congestion and enlargement with peripheral hepatic necrosis. The rate of isolation of ***Clostridial*** spp. recovered from 756 rabbits, feed and water samples was 311 (41.13%). Only 135 (41.03%) out of 329 examined rabbits was positive for ***Clostridial*** spp. that was distributed as the following; 109 (80.74%) exhibited single ***Clostridial*** spp., 4 (2.96%) showed mixed infection with more than one ***Clostridial*** spp. and 22 (16.29%) were un-typable. From 135 positive ***Clostridial*** spp.; ***Clostridium perfringens (C. perfringens***), ***C. tertium***, ***C. sporogenes***, ***C. bifermentans***, ***C. septicum*** and ***C. difficile*** were recovered as 35 (25.92%), 32 (23.70%), 19 (14.07%), 14 (10.37%), 5 (3.70%) and 4 (2.96%), respectively. Mixed types **(*C. perfringens*** and ***C. tertium)*** were represented as 2 (1.48%), ***(C. perfringens*** and ***C. sporogenes)*** 1 (0.74%) as well as **(*C. perfringens*** and ***C. difficile)*** 1 (0.74%). Seven (18.42%) out of 38 examined feed and water samples was positive for ***Clostridial*** spp. where ***C. perfringens*** was the only ***Clostridial*** spp. that isolated at a rate of 6/19 (31.57%) from feed and 1.0/19 (5.26%) from water samples. The distribution of ***Clostridial*** spp. among surveyed rabbit's farms at different Egyptian governorates was detected. On histopathological examination, fibrosis in the portal area of liver as well as infiltration with inflammatory cells, and also diffuse inflammatory cells, oedema and necrosis was observed in intestines.

[Khelfa D. E. -D. G., Wafaa A. Abd El-Ghany and Heba M. Salem **Recent Status of *Clostridial* Enteritis Affecting Early Weaned Rabbits in Egypt**. *Life Sci J* 2012;9(4):2272-2279] (ISSN:1097-8135). http://www.lifesciencesite.com. 337

Keywords: Weaned rabbits; Enteritis; ***Clostridium*** species; Egypt.

1. Introduction:

Rabbits industry is one of the small livestock industries that play a considerable role in solving the problem of meat shortage in developing countries (Lepas *et al.*, 1997). The domestic rabbits when compared with other livestock animals are characterized by early sexual maturity, high prolificacy, relatively short gestation length, short generation interval, high productive potential, rapid growth, good ability to utilize forages and fibrous plant materials, more efficient feed conversion, lower cost per breeding female and its profitability for small-scale production system (Cheecke, 1986; Finzi and Amici, 1991).

Enteritis in rabbits mainly after weaning is the major cause of economic losses in commercial rabbitaries as it induces high mortalities about 27-50% at 5-7 weeks of age (Scharmann and Wolff, 1985). The epizootic rabbit enteropathy (ERE) has become a threat to the industry as it can cause between 20 - 70% mortality and up to 100% morbidity in European rabbit commercial farms (de Blas *et al.*, 2012).

Many causes are claimed in induction of enteritis in rabbits as ***Clostridium*** species (spp.), ***Escherichia coli***, ***Staphylococcus aureus***, ***Salmonella*** spp. and ***Vibrio*** spp. (Hara-Kudo *et al.*, 1996). ***Clostridium*** spp. are the most important one (Szemeredi *et al.*, 1983) as they adversely affecting rabbit's industry all over the world (Diab *et al.*, 2003).

Clostridial organisms are widely distributed pathogens commonly isolated from the environment and the gastrointestinal tract of rabbits (Hein and Timms, 1972). ***Clostridium perfringens (C. perfringens), C. piliformis, C. spiroforme*** and ***C. difficile*** are the most common bacterial causes of enteritis complex in rabbits (Tzika and Saoulidis,

2004). ***C. perfringens*** is one of the most widely distributed and the most dangerous spp. members of the genus ***Clostridium*** that affecting rabbit's farms (Timoney *et al.,* 1988). ***C. perfringens*** vegetative cells cause both histotoxic infections (e.g. gas gangrene) and diseases originating in the intestines (e.g. hemorrhagic necrotizing enteritis or lethal enterotoxemia) (Menglin *et al.,* 2011). Toxigenic types of ***C. perfringens*** are significant causative agents of enteric disease in domestic animals (Miyashiro *et al.,* 2009).

Severe ***C. difficile*** toxin-induce rabbit enteritis which characterized by exuberant intestinal tissue inflammation, epithelial disruption and diarrhea (Cirle *et al.,* 2012).

From the above mentioned, this work was designed to through light on the recent status of ***Clostridial*** enteritis affecting early weaned rabbits and the role of feed and water in transmission of such infection at different Egyptian governorates.

2. Materials and Methods

Field diagnosis of Clostridial enteritis among examined weaned rabbit farms at different Egyptian governorates:

Field diagnosis of Clostridial enteritis based on clinical examination including flock history and palpation of the examined rabbits for detection of abnormal intestinal contents and excessive gases (bloat) was carried out according to Ivanics et al., (1982).

Sampling:

It was applied as Cruickshank et al., (1975). Equal two samples (rectal swabs, liver and intestine) from a total of 718 samples expressing 329 examined rabbits (95 apparently healthy, 204 clinically affected and 30 freshly dead ones) were collected from each examined rabbits in separate sterile bag with serial number corresponding to each flock. Moreover, equal number (19) of feed and water samples were also collected from each examined farm in identified and labeled sterile plastic cups with a serial number corresponding to each flock. All rabbit's samples were rapidly transferred to the laboratory on ice for isolation of Clostridium spp. One sample was used for isolation of C. perfringens, whereas the other one was used for isolation of spp. other than C. perfringens.

Isolation of Clostridium spp.

The method was adopted as Smith and Holdman, (1968). Each sample was transferred aseptically into two separate sterile test tubes containing cooked meat media. The media were previously heated in boiling water bath for 10 min. To drive off any dissolved oxygen and then rapidly cooled in a cold bath just prior to their inoculation with the samples. Immediate inoculation of samples was done to ensure that cultures were placed under anaerobic conditions. One of the inoculated tubes was heated at 80º C for 10 min. In a water bath with a depth of water more than the level of the tube content to eliminate non spore forming aerobes and allow heat resistant spore former Clostridium spp. to grow, while the other tube was left unheated. Both heated and unheated inoculated tubes were incubated at 37º C for 48 hrs. under anaerobic conditions (Gas Pack Jar). A loopful from unheated tubes was then streaked on neomycin sulfate 10% sheep blood agar plate, while the other loophole was taken from heated culture and streaked onto 10% sheep blood agar plates. All the inoculated plates were incubated anaerobically at 37ºC for 24-48 hrs. Sub-culturing of the identified culture was restored in cooked meat media and then kept in the refrigerator for purification and further identification.

Identification of Clostridial isolates:

Colonial morphology:

Suspected different Clostridial colonies were examined morphologically (Vaikosen and Muller, 2001).

Microscopical examination:

Smears from suspected Clostridial colonies were stained with Gram's stain and examined microscopically for detection of morphological characters of Clostridial microorganisms (Cruickshank et al., 1975).

Biochemical reactions:

Suspected purified Clostridial isolates were identified biochemically using catalogs, sugar fermentation, gelatin liquefaction, indole, urease, lecithinase and meat digestion as well as motility test according to the schemes of Koneman et al., (1992) and Macfaddin, (2000).

Histopathological examination:

Tissue samples were taken from livers as well as intestines of naturally infected weaned rabbits showed characteristic Clostridial post-mortem lesions and then processed for histopathological examination according to Banchroft et al., (1996).

3. Results and Discussion

Rabbit's industry and production have been developed and expanded all over the world to fill the gap between available and required animal protein for human being. Great attention is directed to the

diseases causing economic losses to this industry from time to time (Finzi and Amici, 1991).

The history of examining rabbit's farms at different Egyptian governorates revealed that, the examined breeds were floundering, Belgian, French, Erks, Hi-plus, Native, New Zealand, Chinchilla, Gabali and Moshtohor with ages ranged from 3 - 9 weeks. The total number of rabbits per farm ranged from 35- 800 rabbits, however, the number of dead rabbits at each examined farm at day of examination ranged from 1.0 to 20. The system of housing of examined rabbits was battery and ground breeding systems. All examined rabbits were fed on commercial ration. Most of examined flocks were vaccinated with rabbit haemorrhagic disease virus vaccine and formalized polyvalent rabbit pasteurellosis vaccine. Also, antibacterial agents were used on some examined farms.

The most commonly observed clinical signs on examined early weaned rabbits at the time of visiting the farm were severe bloat associated with offensive odour doughy brownish diarrhea (Fig. 1) that soil the regions around anuses and hind quarters, inability to walk, depression and ruffled fur. Similar signs on naturally infected rabbits with *Clostridial* organisms were observed by Baskerville *et al.,* (1980); Ivanics *et al.,* (1982); Nagi *et al.,* (1988); Hunter *et al.,* (1992) and Mostafa (1992).

Palpation of the clinically affected rabbits exhibited pain response on palpation of their abdomens which were distended with gases.

The recorded post-mortem lesions of *Clostridial* enteritis in the examined freshly dead rabbits were severe enteritis, typhlitis, ballooning with offensive odour doughy brownish or bloody stained contents mixed with gases, different degrees of necrosis and hemorrhages of the mucousa and the mesenteric blood vessels were engorged with blood (Fig. 2 and 3). Similar findings were recorded by Prescott, (1977). The liver showed congestion, enlargement with sub-capsular hemorrhages, necrosis especially at its margins (Fig. 4) and friability as well as distended gall bladder. Kunstyr *et al.,* (1975) found similar hepatic lesions in rabbits infected with *Clostridial* enteritis. The kidneys were congested and enlarged (Fig. 5) and the urinary bladder was distended with urine (Fig. 6). Our results about kidney lesions resembled these recorded by Baskerville *et al.,* (1980); Nagi *et al.,* (1988); Abdel-Rahman *et al.,* (2006) and Shi Xi Shan *et al.,* (2008) in dead rabbits with different *Clostridial* enteritis.

The results of isolation rate of *Clostridial* spp. from examined rabbit's farms at different Egyptian governorates were observed in Table (1). The results demonstrated that a total of 311 (41.13%) *Clostridial* spp. was isolated from 756 examined samples which recovered from 329 surveyed rabbits as well as 38 feed and water samples. The obtained isolation rate (41.13%) was higher than McDonal and Duncan, (1975) 37.6%, Szemeredi *et al.,* (1983) 39.0% and Mostafa, (1992) 35.2%. This difference in the isolation rate between this study and the others may be related to the difference in the date of surveillance, season, locality, feeding and housing system.

A total of 135 *Clostridial* isolates recovered from 329 examined rabbits (41.03%) were subjected for spp. identification on the basis of colonial appearance on blood agar, microscopical appearance and biochemical identification. The prevalence of *C. perfringens* and *Clostridial* spp. other than *C. perfringens* isolated from examined rabbits was illustrated in Table (2). The results showed that 109 (80.74%) out of 135 isolated *Clostrdial* organisms exhibited single infection, but 4 (2.96%) showed mixed infection with more than *Clostridial* spp., whereas 22 (16.29%) were un-typable spp.

C. perfringens constituted the higher incidence (25.92%), followed by *C. tertium* (23.70%), *C. sporogenes* (14.07%), *C. bifermentans* (10.37%), *C. septicum* (3.70%), *C. difficile* (2.96%) and untypable (16.29%) spp. Nearly similar finding was reported by Mostafa, (1992) who isolated *C. perfringens* in percentage of 23%. However, higher incidences were recorded by Lee *et al.,* (1991) 76.5 %, Abdel-Rahman *et al.,* (2006) 39.3% and Heba, (2010) 86%. That difference between us and others may be attributed to the state of examining rabbits, as *C. perfringens* is one of the most widely distributed and the most dangerous spp. members of the genus *Clostridium* that affecting rabbit's farms (Timoney *et al.,* 1988).

The incidence of *C. tertium* and *C. difficile* in this study were 23.70 and 2.96%, respectively which were nearly comparable to that recorded by Hughes *et al.,* (1983) who recovered *C. tertium* and *C. difficile* in percentages of 25.7 and 2.5%, respectively. Also, Bano *et al.,* (2008) isolated *C. difficile* from the content of the small intestine and impacted caecum of 319 diseased rabbits and 80 apparent healthy ones. On the other hand, the obtained results are disagree with that of Mostafa, (1992) who recorded lower incidence rate of *C. tertium* (0.83%) and *C. difficile* (0.90%) from 358 diseased and apparently healthy surveyed rabbits. In addition, higher percentage (10%) of *C. difficile* isolation from dead rabbits with intestinal pathological lesions was reported by El-Rahman and Atwa, (2006). This disagreement may be owing to the hazardous adopted hygienic measures and the system of housing in the surveyed farms.

This work succeeded in isolation of *C. sporogens*, *C. bifermentans* and *C. septicum* in rabbits which

causing *Clostridial* enteritis of the surveyed early weaned rabbits. Similarly, Peeters *et al.*, (1986) isolated *C. sporogens*, *C. bifermentans* and *C. septicum* from rabbit's intestine.

Mixed types between *C. perfringens* and spp. other than *C. perfringens* were *C. perfringens* and *C. tertium* (1.48%), *C. perfringens* and *C. sporogenes* (0.74%) as well as *C. perfringens* and *C. difficile* (0.74%).

Table (3) declared that 7 out of 38 (18.42%) examined feed and water samples was positive for *Clostridial* spp. where *C. perfringens* was the only *Clostridial* spp. that isolated at a rate of 6/19 (31.57%) from feed and 1.0/19 (5.26%) from water samples. No *Clostridial* spp. other than *C. perfringens* was recovered from feed and water. Our results agree with that recorded by Heba, (2010) who recovered *C. perfringens* from feed and water of rabbits.

The distribution of different types of isolated single and un-typable *Clostridial* spp. among surveyed rabbit's farms at Port-Said, Giza, Cairo, Beni Suef, El-Fayoum, El-Qaliubiya, El-Sharkia and El-Menoufia governorates was represented in Table (4). Other Egyptian researchers like Heba, (2010) found that the incidence of *C. perfringens* infection in Giza governorate was 86%, meanwhile, El-Rahman and Atwa, (2006) demonstrated that the incidence rates of *C. perfringens* were 30, 18 and 10% from the intestines, livers and fecal samples, respectively from 300 diseased and died 4-12 weeks old rabbits in El-Menoufia governorate. Moreover, Abdel-Rahman *et al.*, (2006) detected that the incidence rate of *C. perfringens* that isolated from 140 rectal swabs from apparently healthy, diarrheic and dead weaned rabbits was 39.30% in El-Menia and Assuit governorates. This difference in spp. isolation may be attributed to the difference in governorates, season, state of examined rabbits and the usage of antibiotics.

The histopathological examination of livers collected from freshly dead weaned rabbits showed fibrosis of the portal area with newly formed bile ducts (Fig. 7. A), associated with diffuse kupffer cells proliferation and inflammatory cells infiltration in between the hepatocytes (Fig. 7. B). Severe congestion was observed in the central vein while the surrounding hepatic parenchyma was brown pigmented material (Fig. 7. C). Prescott, (1977) and Heba, (2010) found similar histopathological alterations in dead rabbits due to *Clostridial* organisms. The findings of small intestine revealed necrosis involving the mucosal layer with desquamation of the lining epithelium while the underlying sub-mucosa showed oedema, inflammatory cells infiltration and congested blood vessels and capillaries (Fig. 7. D, E and F). Microscopic lesions observed in the large intestine were diffuse mucosal necrosis and ulceration all over the lining epithelium with inflammatory cells infiltration in the lamina propria (Fig. 7. G). The same microscopic changes in the small and large intestine of *C. perfringens* naturally infected dead rabbits were observed by Badagliacca *et al.*, (2010) and Francisco *et al.*, (2012).

Table (1): The isolation rate of *Clostridial* spp. from surveyed rabbit's farms at different Egyptian governorates

Governorate	No. of examined farms	Examined samples			Isolation of *Clostridial* spp.			
					Clostridial positive samples		*Clostridial* negative samples	
		Swabs and organs	Feed and water	Total	No.	%	No.	%
Port Said	2	78	4	82	30	36.58	52	63.42
Giza	4	200	8	208	83	39.9	125	60.10
Cairo	2	32	4	36	16	44.44	20	55.55
Beni Suef	2	36	4	40	17	42.5	23	57.5
El-Fayoum	3	128	6	134	59	44.02	75	55.97
El-Qaliubiya	4	120	8	128	55	42.97	73	57.03
El-Sharkia	1	48	2	50	20	40	30	60
EL-Menoufia	1	76	2	78	31	39.74	47	60.26
Total	19	718	38	756	311	41.13	445	58.87

Table (2): Prevalence of *C. perfringens* and *Clostridial* spp. other than *C. perfringens* isolated from examined rabbits at different Egyptian governorates.

No. of examined rabbits	*Clostridial* spp.	No. of identified *Clostridial* spp.	%
329	Single types	109	80.74
	C. perfringens	35	25.92
	C. tertium	32	23.70
	C. sporogens	19	14.07
	C. bifermentans	14	10.37
	C. septicum	5	3.70
	C. difficile	4	2.96
	Mixed types	4	2.96
	C. perfringens + C. tertium	2	1.48
	C. perfringens + C. sporogenes	1	0.74
	C. perfringens + C. difficile	1	0.74
	Un-typbable	22	16.29
Total		135	

Table (3): Prevalence of *C. perfringens* isolated from feed and water samples in examined rabbit's farms at different Egyptian governorates

Type of sample	No. of samples	Recovered C. perfringens	
		No.	%
Feed	19	6	31.57
Water	19	1	5.26
Total	38	7	18.42

Table (4): Distribution of single and un-typable *Clostridial* spp. among surveyed rabbit's farms at different Egyptian governorates

Governorate	No. of examined farms	*C. perfringens*	*C. tertium*	*C. sporogenes*	*C. bifermentans*	*C. septicum*	*C. difficile*	Un-typable *Clostridial* spp.
Port Said	2	3	5	1	2	-	1	9
Giza	4	12	10	7	3	1	1	2
Cairo	2	4	1	-	1	2	-	4
Beni Suef	2	3	3	1	-	1	1	2
El-Fayoum	3	7	4	6	5	-	1	1
El-Qaliubiya	4	3	2	2	1	-	-	2
El-Sharkia	1	2	3	1	2	-	-	1
El-Menoufia	1	1	4	1	-	1	-	1
Total	19	35	32	19	14	5	4	22

Fig. (1): A rabbit shows severe bloat and doughy brownish diarrhea.

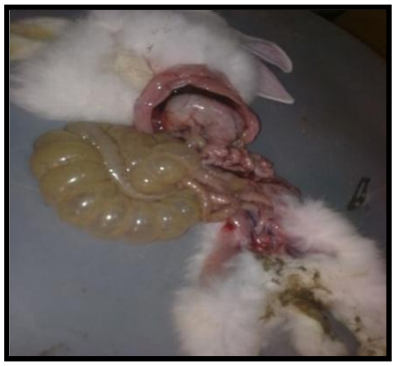

Fig. (2): Large intestine of rabbit exhibited ballooning and filled with gases

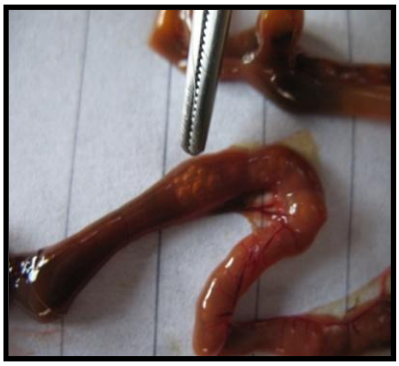

Fig. (3): Small intestine of a rabbit shows different degrees of enteritis, the intestine distended with gases and the mesenteric blood vessels are engorged with blood**.**

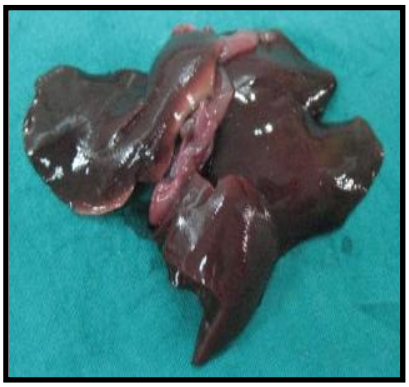

Fig. (4): A rabbit's liver reveals congestion, enlargement, sub-capsular hemorrhage and necrosis especially at liver's margins (arrow)

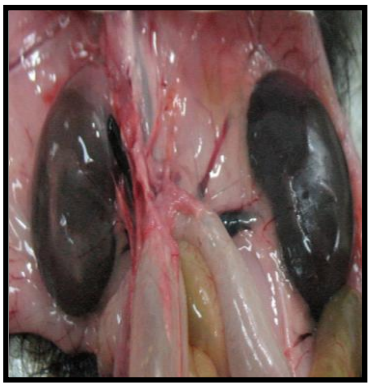

Fig. (5): Congested and enlargement of a rabbit's kidneys.

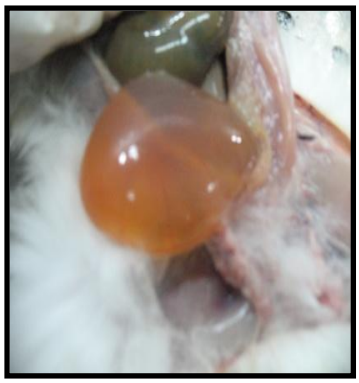

Fig. (6): A rabbit with severely distended urinary bladder with urine.

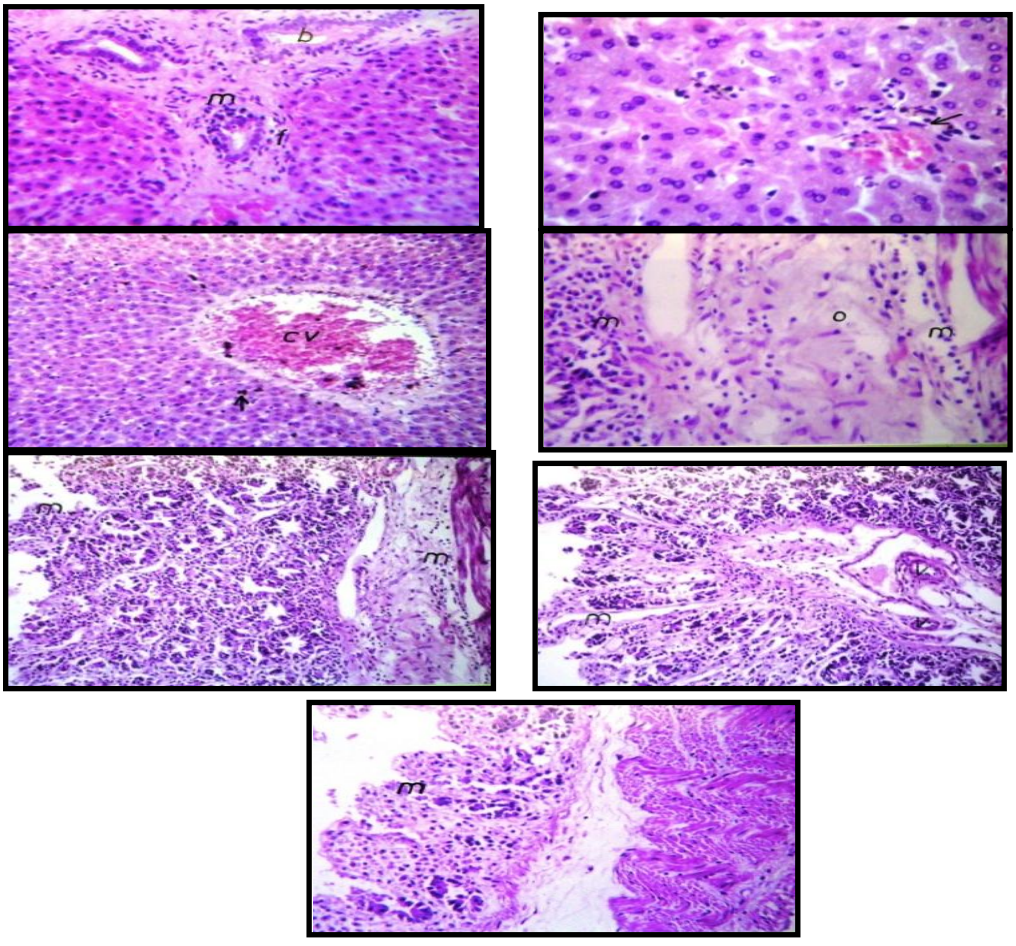

Fig. (7): The histopathological findings are as follow; (A): Liver of a rabbit showing fibrosis in the portal area (f) with multiple newly formed bile ducts (b). **H&E X 64, (B):** Liver of a rabbit showing diffuse kupffer cells proliferation (arrow) with inflammatory cells infiltration in between the hepatocytes. **H&E X 80, (C):** Liver of a rabbit showing severe congestion in central vein (cv) with diffuse brown pigmented material (arrow) in between hepatocytes. **H&E X 40, (D):** Small intestine of a rabbit showing diffuse inflammatory cells infiltration (m) and oedema (O) in submucosal layer. **H&E X 40, (E):** Small intestine of a rabbit showing diffuse inflammatory cells infiltration (m) and oedema in submucosal layer. **H&E X 80, (F):** Small intestine of a rabbit showing mucosal necrosis (m) with oedema and congestion in blood vessels (v) of submucosa. **H&E X 40** and **(G):** Large intestine of a rabbit showing necrosis in the mucosal layer (m) and ulceration with inflammatory cell in lamina propria. **H&E X 64.**

Conclusion

Finally, it could be concluded that *Clostridial* spp. are incriminated in the induction of enteritis problem in rabbits with high percentages. There are different types of *Clostridial* spp. circulating in weaned rabbit's farms at different Egyptian governorates, so it should be taken into consideration during the research work. In addition, contamination of feed and water with *C. perfringens* plays a prominent role in the fulmination of this problem, so it is recommended developing of new management strategies including testing feed ingredients periodically for spore contamination and also checking the water sources for monitoring management programs.

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10/12/2012