Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 5 | Issue : 01 | Jan-Mar. | 2015
Pages: 252-261

Field and Laboratory Diagnosis of *C. perfringens* Enteric Infection among Rabbit Flocks in Egypt

[1]Khelfa D.G., [1]Madian K., [3]El-Meneisy A. A., [2]Faten F. Mohammed and [1]Heba M. Salem

[1]Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
[2]Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
[3]Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

ABSTRACT

A surveillance study for diagnosis of *Clostridium perfringens* (*C. perfringens*) enteritis affecting early weaned rabbits was carried out on sixteen rabbit flocks, during the period 2012 - 2014. Diagnosis based on flock history, clinical signs, post-mortem lesions and histopathological examinations as well as isolation and identification of *C. perfringens*. Two hundred and sixty seven rectal swabs from diseased rabbits suspected to be infected with *C. perfringens*, moreover tissue samples from intestine and liver of 48 freshly dead rabbits were aseptically collected for isolation and identification of *C. perfringens* by cultural and biochemical characterization. A tissue specimens from small, large intestine, liver, spleen and kidney from each examined freshly dead rabbits were processed for histopathological examination. The results of flock history revealed that, examined breeds were (Baladi, French, California, Bauscat, Chinchilla, New-Zealand and Dutch) with age ranged from 3-9 weeks and mortality rate 12-60%. Observed clinical signs were severe diarrhea and bloat. Post-mortem lesions showed different degrees of enteritis and intestinal content usually mixed with gases. Liver showed congestion, enlargement and gall bladder distended with bile. Kidney showed congestion, enlargement and friability. Histopathological alterations were necrohemorrhagic enteritis in small and large intestine, portal congestion, periportal edema, sinusoidal dilatation and hepatic hemorrhage in liver, depletion and necrosis of lymphoid elements in spleen and degeneration in the tubular epithelium, vacuolization of epithelial lining of renal pelvic with perivascular edema in kidney. The isolation rates and percentages of recovered *C. perfringens* were from rectal swabs (118/267) 44 %, intestine (40/48) 83% and liver (9/48) 18%. *C. perfringens* enteric infection could be detected in a higher rate in both autumn & winter seasons especially at 3-4 weeks. Most affected breeds were Baladi, followed by French, California, Bauscat, Chinchilla, New-Zealand and Dutch breeds with a percentages 83, 80, 75, 70, 50, 35 and 20%, respectively. Incidence of *C. perfringens* was higher in mixed breeds more than pure breeds, females (99/105) 94% more than males (68/210) 32% and rabbits reared on ground more than those reared on battery system. The isolation rate of *C. perfringens* in rabbit flocks used antibacterial drugs was high 11flocks out of 16 (68%). All isolated *C. perfringens* strains were toxinogenic to Swiss mice.

Key words: Weaned rabbits, Enteritis, *C. perfringens*, Histopathology, Egypt.

Introduction

Rabbits 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. Clostridial enteritis is a major cause of economic losses in commercial rabbitaries especially at weaning age Finzi and Amici, (1991).

C. perfringens produce toxins in the intestine which is an important cause of enteritis, enterotoxaemia and fatal to livestock. It causes high mortalities (27-50%) at five to seven weeks of age and may reach 100% in unvaccinated herd (Scharmann and Wolff, 1985; Uzal *et al*., 2003; Youhanna and songer, 2006; Cocchi *et al*., 2008; Sting, 2009; Agnoletti, 2012 and Nagahama *et al*., 2015). Saggiorato *et al*., 2008 isolated *C. perfringens* with a high percentage from weaned rabbits with enteric diseases in Italy, France and Spain. In Egypt, the incidence of *C. perfringens* enteric infection among weaned rabbits was 39, 88, 78, 79.9 and 62.9% as recorded by Abdel-Rahman *et al*., 2006; El-Bakrey, 2009; Lebdah and Shahin, 2011; Khelfa *et al*., 2012 and Nashwa *et al*., 2014, respectively.

Materials and Methods

Field diagnosis of clostridial enteritis among weaned rabbits:

Corresponding Author Heba Mohammed Salem, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Giza -12211, Egypt
E-mail : dr.hebasalem@cu.edu.eg

Field diagnosis of clostridial enteritis based on recording flock history of examined rabbit flocks including (number of weaned rabbits/flock, observed clinical signs, mortality rate (no. of dead rabbits/total no. of rabbit, based on flock records), age, breed, housing system, previous vaccination, previous medication and season) as well as clinical examination by palpation and inspection of diseased weaned rabbits (Ivanics *et al.,* 1982).

Sampling:

Two hundred and sixty seven rectal swabs from diseased rabbits with enteric problem and tissue samples from liver and intestine of 48 freshly dead rabbits were aseptically collected in separate sterile bag with serial number corresponding to each flock. All samples were rapidly transferred to the laboratory on ice for isolation of *C. perfringens* (Cruickshank *et al*., 1975).

Isolation of C. perfringens:

Each sample was transferred aseptically into sterile test tubes containing cooked meat media and incubated anaerobically at 37 ºC for 24-48 hrs. A loopful from tube was streaked on neomycin sulfate 10% sheep blood agar plate and incubated anaerobically at 37 ºC for 24-48 hrs. Colonies was inoculated on cooked meat media and then kept in the refrigerator for further identification (Smith and Holdman, 1968).

Identification of C. perfringens isolates:

Colonial morphology

Suspected *C. perfringens* colonies were morphologically examined (Vaikosen and Muller, 2001).

Microscopical examination:

Smears from suspected *C. perfringens* colonies were stained with Gram's stain and microscopically examined (Cruickshank *et al.,* 1975).

Biochemical reactions

Purified suspected *C. perfringens* isolates were biochemically identified using catalase, sugar fermentation, gelatin liquefaction, indole, urease and lecithinase tests according to the schemes of (Koneman *et al.,* 1992 and Macfaddin, 2000).

Determination of toxigenic strains of C. perfringens:

Accomplished through intravenous inoculation in Swiss mice (Mariano *et al*., 2007).

Histopathological examination:

Tissue specimens including liver, kidneys, small and large intestines were collected from each 48 freshly dead rabbit processed for histopathological examination (Banchroft *et al.,* 1996).

Results

Flock history of examined rabbit flocks were illustrated in table (1).Observed clinical signs of weaned rabbits were depression, ruffled fur, inability to walk (Fig.1,a), bloat (Fig.1,b) and doughy brownish to bloody stained diarrhea that soil the hind quarters (Fig.1,c). Palpation of affected rabbits with distended abdomen revealed pain expression response.

P.M. examination showed that the small intestine in some cases was ballooned (Fig.1, d) and had variable degrees of enteritis (Fig.1, e). All rabbits had variable amounts of white mucoid, brownish, hemorrhagic offensive odour intestinal contents (Fig.1, h).The mesenteric blood vessels were engorged with blood (Fig.1, g). The colon and cecum were ballooned with different degrees of enteritis (Fig.1, I & j). The liver showed congestion (Fig.1, k), enlargement, sub-capsular hemorrhages and friability, while the gall bladder was distended with bile (Fig.1, L). The kidneys were congested, enlarged and friable (Fig.1, m). Urinary bladder was usually distended with yellowish white contents and the bladder blood vessels were engorged with blood (Fig. 1, n). Spleen in some cases was enlarged and congested, stomach was usually feed impacted with

offensive odour (Fig.1, o) and in some cases, gastric mucosa has variable degrees of hemorrhages and congestion (Fig.1, p).

The histopathological alterations of small intestine denoting necrohemorrhagic enteritis that involves intestinal mucosa with less reaction in the remained intestinal layers, the lesion characterized by blunting of intestinal villi, desquamation of intestinal mucosa, mononuclear inflammatory cells infiltration mainly macrophages mixed with RBCs associated with edema and dilatation of blood capillaries within lamina propria in addition to pseudomembranous enteritis that characterized by fusion of intestinal villi, marked destruction of intestinal mucosa that covered by eosinophilic structurless necrotic tissue admixed with fibrin, cell debris and inflammatory cells with free RBCs exudation, the lamina propria showing intense edema, inflammatory cells infiltration mainly heterophiles and macrophages with dilatation of blood capillaries (Fig.2), While large intestine represented picture of necrohemorrhagic typholocolitis that characterized by necrosis of intestinal mucosa that covered by necrotic debris, desquamated epithelium and inflammatory cells with necrosis of colonic glands, the lamina propria showing congestion of blood vessels, interstitial edema and macrophages infiltration with RBCs exudation in addition to necrotizing type of enteritis, the lesion was characterized by marked necrosis of intestinal mucosa with sever edema of lamina propria and submucosa with inflammatory cells infiltration with dilatation of blood capillaries (Fig.3). The microscopic examination of liver revealed portal congestion, periportal edema, sinusoidal dilatation and hepatic hemorrhage (Fig.4). The most prominent histopathological alterations in the spleen were congestion of splenic sinusoids associated depletion and necrosis of lymphoid elements involving the lymphoid follicles (Fig.5). There were few renal histopathological alterations. Renal lesions restricted into congestion of interstitial blood vessels in renal cortex and medulla with mild degree of degeneration involving the tubular epithelium, in addition to vacuolization of epithelial lining of renal pelvic with perivascular edema (Fig.6). The histopathological findings proved that, all examined rabbits died from *C. perfringens* enteric infection.

Table 1: Flock history of surveyed rabbit flocks

Flock no.	No. of weaned rabbits /flock	Season	Mortality rate	Age (week)	Breeds/flock	Housing	Pervious vaccination	Pervious medication	Observed clinical signs
1	520		40%	3-5	New- Zealand and California	B	*	Pan terramycin (inj.) 0.5 cm/rabbit	=
2	2600	autumn	60%	6-8	New- Zealand and Bauscat	B	*	-	=
3	60	autumn	20%	5-8	New- Zealand and Baladi	B	*	Oxtetracyclin 2cm/litre/5days	=
4	50	autumn	20%	6-8	New- Zealand and French	B	*	Flagyl 1cm/litre/3days	=
5	800	autumn	60%	5-7	New- Zealand	B	*	Enrofloxacin (inj.)0.5 cm/rabbit	=
6	690	autumn	50%	3-6	Bauscat, California and French	B	*	+ Neomycin sulphate 1cm/litre/3days	=
7	1370	autumn	30%	3-4	New- Zealand, Dutch, Bauscat and California	B	*	Chlortetracyclin 1cm/litre/5days	=
8	2350	winter	20%	3-5	New- Zealand	B	*	Hepadex1cm/litre/3days Neomycin sulphate 1cm/litre/3days, Amoxicillin 1cm/litre/3days	=
9	220	winter	15%	6-8	New- Zealand , Chinchilla, Dutch, Bauscat and California	B	*	Sulfadimidin and Oxytertracyclin 2cm/litre/5days	#
10	400	winter	12%	7-8	New- Zealand , French and Baladi	B	*	Pan terramycin (inj.) 0.5 cm/rabbit	=
11	200	winter	30%	4-9	New- Zealand	B	*	-	#
12	350	winter	16%	4-9	New Zealand	B	*	Enrofloxacine(inj.) 0.5 cm/rabbit and Flagyl 1cm/litre/3days	#
13	460	winter	30%	5-8	New Zealand	B	*	-	#
14	130	winter	20%	4-6	Baladi	G	-	Oxytetracycline 1cm/litre/4days	=
15	110	winter	20%	4-8	New- Zealand	B	*	-	#
16	470	winter	30%	4-9	New- Zealand	B	*	-	#
		winter							

B: Batteries breeding. *G: Ground breeding.*
**: The flock received both rabbit viral and bacterial hemorrhagic diseases vaccines.*
=: Diarrhea, off food, ruffled fur and unable to walk.
#: Diarrhea and/or bloat.

Suspected colonies on neomycin sulphate sheep blood agar were rounded, raised, smooth, opaque, glistening, showed double zones of hemolysis. Smears from the colonies stained with Gram's stain for microscopical examination, revealed Gram positive, short bacilli, straight with parallel sides and rounded ends. *Clostridium* isolates were positive with gelatinase activity, lecithinase reaction, sugar fermentation tests (lactose, glucose, sucrose and maltose), while were negative with indole, ureases and catalase tests.

The isolation rate and percentages of recovered *C. perfringens* were from rectal swabs (118/267) 44%, intestine (40/48) 83% and liver (9/48) 18% (table 2). All isolated *C. perfringens* strains were toxinogenic to Swiss mice as it died within few hours up to 24 hrs. after inoculation.

C. perfringens enteric infection could be detected in a higher rate in

both autumn & winter seasons at age 3-4 weeks, followed by 4-5, 5-6, 6-7, 7-8 and 8-9 weeks as seen in (table 3). Most affected breeds were Baladi, followed by French, California, Bauscat, Chinchilla, New-Zealand and Dutch rabbit's (table 4). Higher incidence of *C. perfringens* infection were observed in mixed breeds than pure breeds, females more than males (table 5) and rabbits reared on ground more than those reared on battery system. Although usage of anti-bacterial agents in some examined flock, the isolation rate of *C. perfringens* was high.

Table 2: Isolation percentages of *C. perfringens* recovered from examined rabbit flocks

Flock no.	Examined weaned rabbits									Total no. of examined samples	Total +ve *C. perfringens* samples	Isolation % in relation to no. of examined samples for each flock
	Diseased			Freshly dead								
	Rectal swabs			Intestine			Liver					
	No.	*C. perfringens* +ve samples	Isolation%	No.	*C. perfringens* +ve samples	Isolation%	No.	*C. perfringens* +ve samples	Isolation%			
1	-	-	-	5	5	100	5	1	20	10	6	60
2	67	29	43.3	-	-	-	-	-	-	67	29	43
3	10	6	60	-	-	-	-	-	-	10	6	60
4	10	7	70	6	6	100	6	2	33.3	22	15	68
5	-	-	-	5	4	80	5	0	0	10	4	40
6	30	22	73.3	10	9	90	10	3	30	50	34	68
7	20	9	45	5	5	100	5	1	20	30	15	50
8	25	10	40	5	3	60	5	0	0	35	13	37
9	-	-	-	3	3	100	3	1	33.3	6	4	66
10	15	8	53.3	-	-	-	-	-	-	15	8	53
11	-	-	-	4	2	50	4	1	25	8	3	37
12	18	6	33.3	-	-	-	-	-	-	18	6	33
13	23	9	39.1	-	-	-	-	-	-	23	9	39
14	30	6	20	-	-	-	-	-	-	30	6	20
15	9	2	22.2	-	-	-	-	-	-	9	2	22
16	10	4	40	5	3	50	5	0	0	20	7	35
Total	267	118	44.1	48	40	83.3	48	9	18.75	363	167	46

+ve: positive.

Table 3: Isolation percentages of *C. perfringens* from weaned rabbits in different ages.

Age (weeks)	3-4	4-5	5-6	6-7	7-8	8-9
No. of examined weaned rabbits	130	80	35	30	18	22
No. of *C. perfringens* positive cases	80	45	20	13	6	3
Isolation%	61	56	57	43	33	13

Table 4: Isolation percentages of *C. perfringens* from weaned rabbits in different breeds.

Examined rabbit breeds	Baladi	French	California	Bauscat	Chinchilla	New-Zealand	Dutch
No. of examined weaned rabbits	36	25	20	57	10	157	10
No. of *C. perfringens* positive cases	30	20	15	40	5	55	2
Isolation%	83	80	75	70	50	35	20

Table 5: Isolation percentages of *C. perfringens* from weaned rabbits in different sex.

Sex of examined rabbits	Males	Females
No. of examined rabbits	210	105
No. of *C. perfringens* positive cases	68	99
Isolation%	32	94

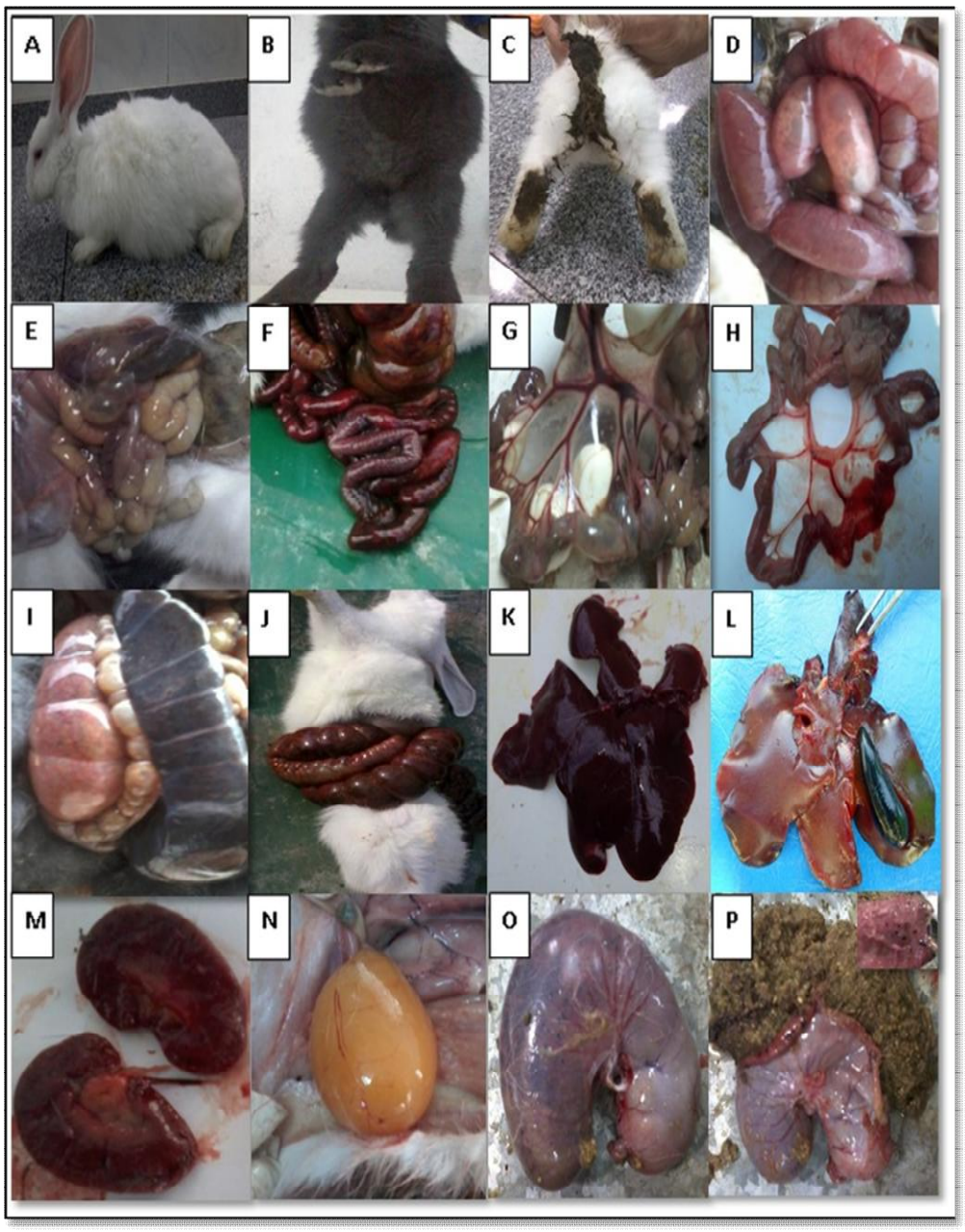

Fig. 1: A: diseased weaned rabbits showing ruffled fur, depression and inability to walk, **B:** weaned rabbits showing bloat, **C:** weaned rabbits showing diarrheic material soiling the hind quarters, **D:** small intestine of weaned rabbit showing ballooning with congestion, **E:** small intestine of weaned rabbit showing different degrees of enteritis, **F:** small intestine of weaned rabbit showing hemorrhagic enteritis, **G:** small intestine of weaned rabbit showing engorgement of mesenteric blood vessels, **H:** opened small intestine of weaned rabbit showing hemorrhagic intestinal content, **I & J:** large intestine of weaned rabbit showing different degrees of ballooning and hemorrhagic enteritis, **K & L:** liver of weaned rabbit showing congestion with engorgement of gall bladder, **M:** kidney of weaned rabbit showing enlargement, congestion and friability, **N:** urinary bladder of weaned rabbit showing distention with yellowish white contents and engorgement of blood vessels, **O & P:** stomach of weaned rabbit showing impaction with feed, congestion with variable hemorrhages on gastric mucosa.

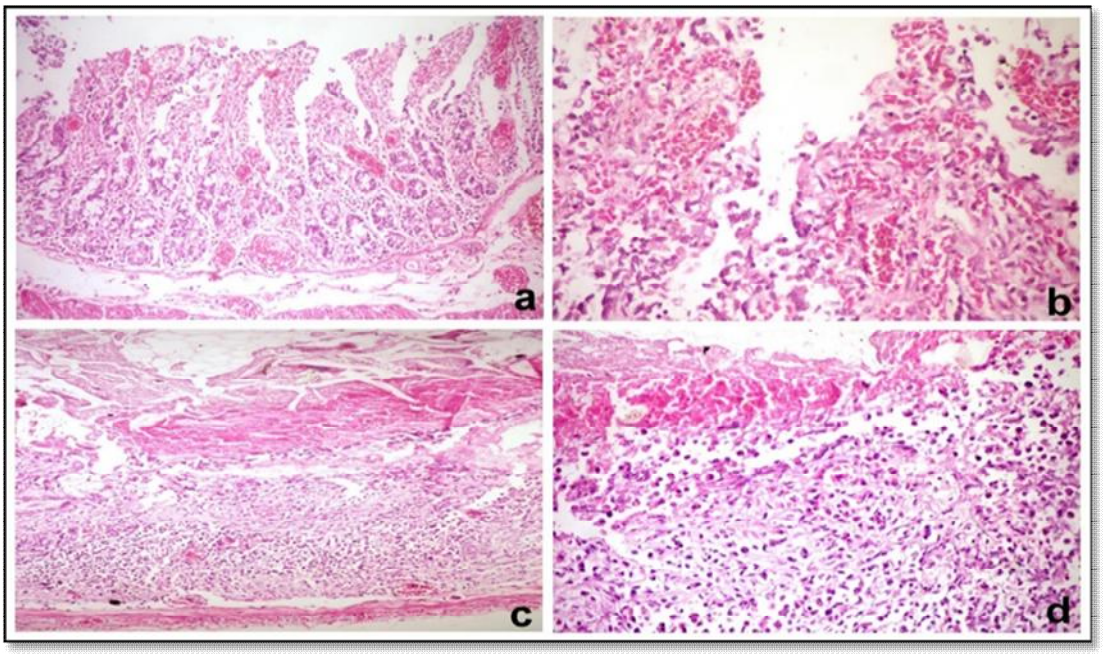

Fig.2: Rabbit small intestine naturally infected by: a: *C. perfringens* showing blunting of intestinal villi with marked congestion of blood capillaries (200X). B: apical necrosis of intestinal villous with edema RBCs exudation mixed with mononuclear cells in the lamina propria (400X). c: showing fusion of intestinal villi and formation of pseudo-membrane covering the necrotic intestinal mucosa with dilatation of blood capillaries of lamina propria (200X).d) higher magnification of the previous, the covering membrane consisted of necrotic debris and fibrin with intense heterophiles and macrophages infiltration and interstitial edema (400X)

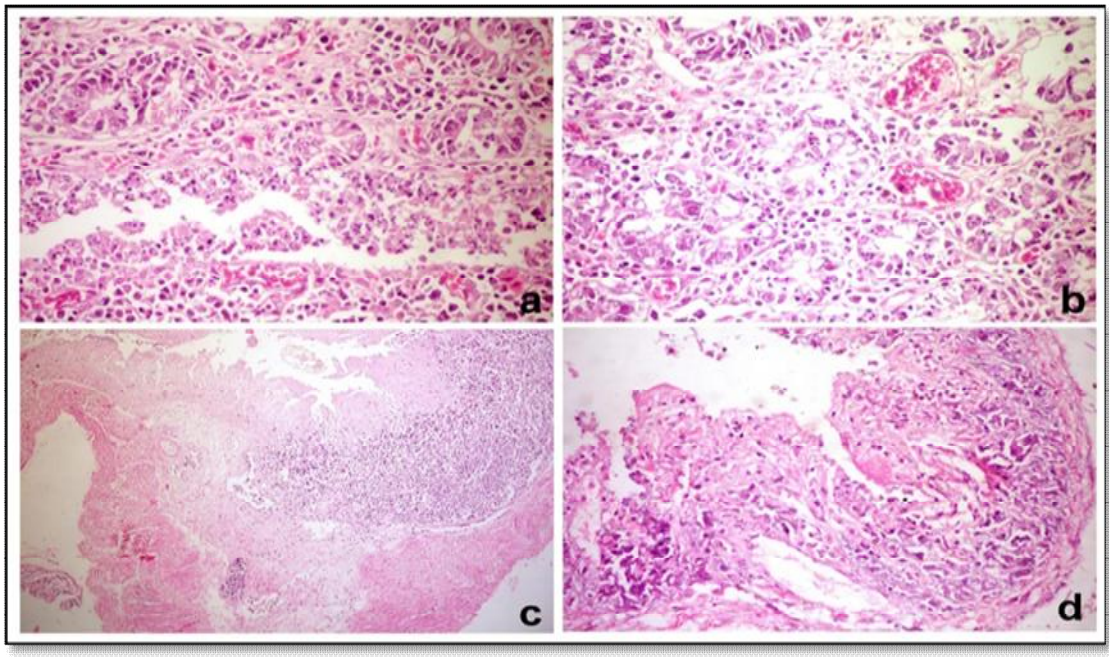

Fig. 3: Rabbit large intestine naturally infected by *C. perfringens* a: showing desquamation of intestinal epithelium that covered by necrotic tissue (400X). b: showing intense inflammatory reaction involving the intestinal propria with interstitial edema, dilated blood capillaries and necrosis of crypt epithelium with nuclear fragments (400X). c: showing intense necrotic and inflammatory reaction involving the intestinal mucosa extending into underlying submucosa that showing edema (100X). d: showing sever necrosis of intestinal mucosa and glands with edema of lamina propria(400X) .

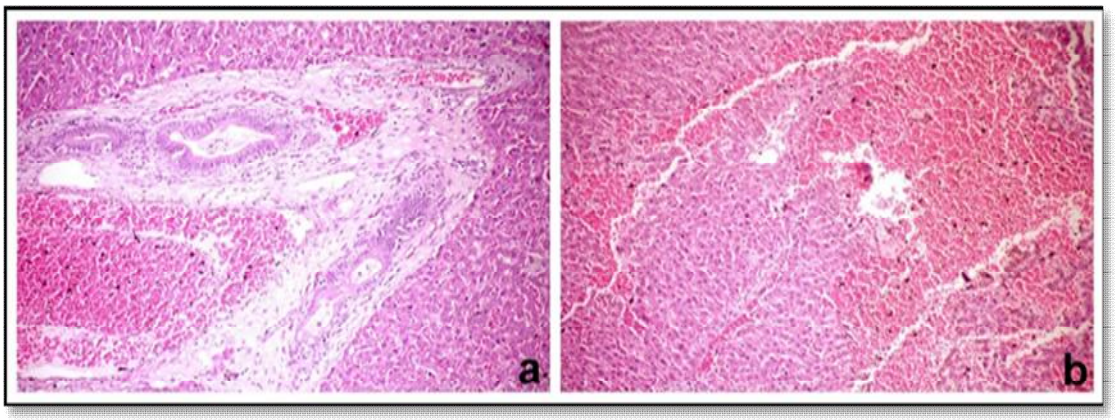

Fig.4: Rabbit liver naturally infected by *C. perfringens* showing sever portal congestion with periportal edema (200X). b: showing hepatic hemorrhage(200X)

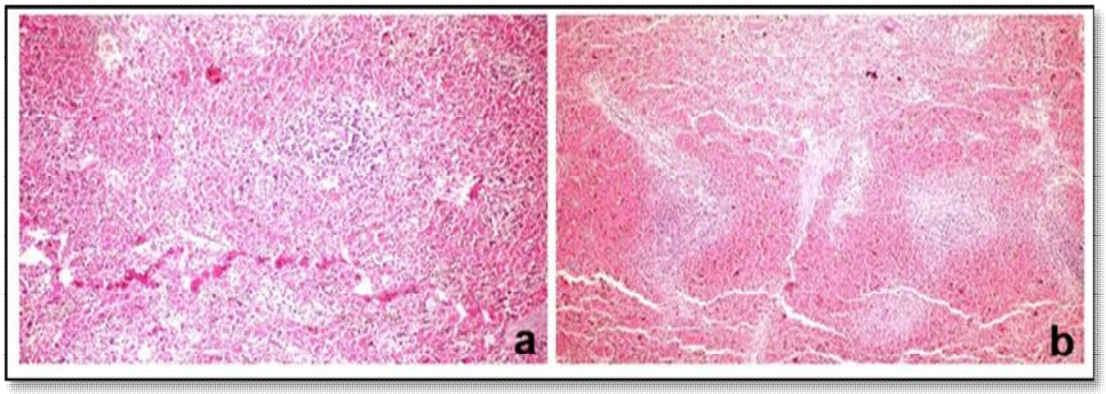

Fig.5: Rabbit spleen naturally infected by *C. perfringens* showing extreme necrosis of lymphoid elements involving the lymphoid follicle that showing atrophy (200X). b: showing sever congestion of splenic sinusoids(100X).

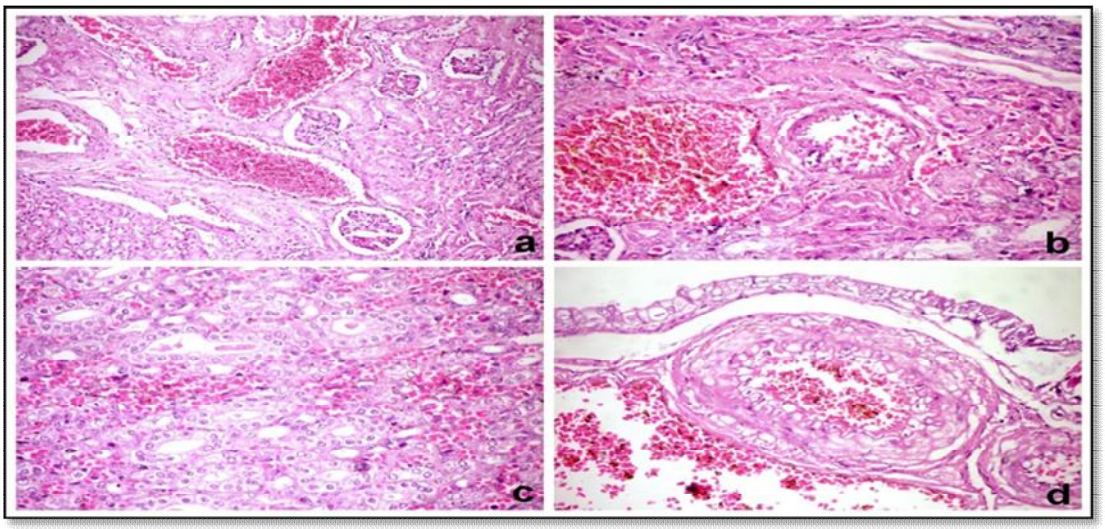

Fig.6: Rabbit kidney naturally infected by *C. perfringens* showing congestion of interstitial blood vessel (400X).b: showing congestion of blood vessels and endothelial swelling of renal arterial wall and individual necrosis of lining tubular epithelium(400X) .c: showing mild congestion of peritubular capillaries with accumulation of eosinophilic proteineous material in individual tubular lumen (400X).d: showing vacuolization of epithelial lining the renal pelvic with extreme congestion , vacuolization of blood vessel wall and swelling of endothelium(400X)

Discussion

Mortality rate in examined rabbit flocks ranged from 20-60%, Scharmann and Wolff, (1985) reported mortalities about 27-50% among weaned rabbits due to *C. perfringens* enteric infection.

The observed clinical signs were depression, ruffled fur, inability to walk, bloat and doughy brownish to blood stained diarrheic material soiled the hind quarters. Similar clinical signs were reported by Baskerville *et al.,* (1980); Ivanics *et al.,* (1982); Nagi *et al.,* (1988); Hunter *et al.,* (1992); El-Deeb, (1992) and Khelfa *et al.,* (2012).

Post-mortem examination of freshly dead rabbits in surveyed flocks revealed that the small and large intestines had variable degrees of enteritis, ballooning, hemorrhages in intestinal mucosa, offensive odour, greenish brown to bloody stained contents mixed with gases and engorged mesenteric blood vessels and this the same as recorded by Prescott, (1977) and Khelfa *et al.,* (2012). The liver usually showed congestion, enlargement, sub-capsular hemorrhages and friability, while the gall bladder was distended. Kunstyr *et al.,* (1975) found similar lesions in liver of rabbits infected with *C. perfringens*. The kidney showed congestion & enlargement and the urinary bladder usually filled with turbid urine. Lesions detected in dead rabbits with *C. perfringens* infection by Baskerville *et al.,* (1980); Nagi *et al.,* (1988); Abdel-Rahman *et al.,* (2006); Shi XiShan *et al.,* (2008) and Khelfa *et al.,* (2012) were resembling to our observed results.

The histopathological alterations of small and large intestine denoting necrohemorrhagic enteritis that characterized by fusion of intestinal villi and marked destruction of intestinal mucosa, while the microscopic examination of liver revealed portal congestion, periportal edema, sinusoidal dilatation and hepatic hemorrhage, the most prominent histopathological alterations in the spleen were congestion of splenic sinusoids associated depletion and necrosis of lymphoid elements involving the lymphoid follicles as well as the renal lesions were restricted into congestion of interstitial blood vessels in renal cortex and medulla with mild degree of degeneration involving the tubular epithelium, in addition to vacuolization of epithelial lining renal pelvic with perivascular edema. Similar histopathological alterations in intestine, liver, spleen and kidney were recorded by Prescott, (1977); Percy *et al*., (1993); Wilber, (1999); Diaa, (2010); Lebdah *et al.,* (2011) and Khelfa *et al.,* (2012).

Isolation percentage was (167/315) 53% from examined rabbits meanwhile, the isolation percentages reported by McDonal and Duncan, (1975); Szemeredi *et al.,* (1983); El-Deeb, (1992) and Abdel-Rahman *et al.,* (2006), were 37.6%; 39.0%; 35.2% and 39.3%, respectively.

The isolation percentage of *C. perfringens* from 363 examined rabbit samples was (167/363) 46% representing (44% from rectal swabs, 83% from intestine and 18% from liver. El-Rhaman and Atwa (2006) recovered *C. perfringens* from faecal samples, intestine and liver in incidences of 36, 70 and 60%, respectively from dead rabbits.

The toxigencity test in Swiss mice and lecithinase activity on egg yolk medium revealed that all 167 *C. perfringens* strains were toxigenic (100%). Meanwhile, El-Deeb, (1992); Abdel-Rahman *et al.,* (2006) and Diaa, (2010) found that the percentage of toxigenic and non-toxigenic *C. perfringens* strains were (68.3 & 31.7%), (81.82 & 18.18%) and (75.4 & 24.56%), respectively, as they examined apparently healthy and diseased rabbits.

The incidence of *C. perfringens* were high during autumn and winter seasons, also El-Bakrey, 2009 found that the incidence of *C. perfringens* infection among weaned rabbits was higher in both autumn and winter than spring and summer seasons and contributed this variation to the intensive rabbit production during autumn and winter seasons as most owners avoid rabbit breeding during spring and summer seasons to prevent losses caused by heat stasis.

The highest incidence of *C. perfringens* enteric infection were observed among weaned rabbits from 3-4 weeks old and decreased by age. Similar results were recorded by Scharmann and Wolff, (1985) and El-Deeb, (1992) who found the highest incidence of *C. perfringens* enteric infection among weaned rabbits about 5-7 weeks and below 2 months, respectively. Also, Finzi and Amici, (1991) noticed that the incidence of *C. perfringens* enteric infection increased in younger ages and decreased by age.

C. perfringens enteric infection could be detected in a higher rate mainly in Baladi, followed by French, California, Bauscat, Chinchilla, New-Zealand and Dutch rabbit's with isolation percentages 83%, 80%, 75%, 70%, 50%, 35% and 20%, respectively. Similar results were recorded by El-Deeb, (1992) who found that the most affected rabbit breeds with *C. perfringens* enteric infection were Baladi, California, Bauscat and New-Zealand rabbits with an incidence 46, 25, 15 and 12%, respectively.

The incidence of *C. perfringens* infection was higher in females than females, same result was recorded by El-Deeb, (1992).

The ground reared rabbits exhibited higher incidence of *C. perfringens* infection than battery system and this result may contributed to the more restricted hygienic measures observed in battery system in addition to the wide spread of *C. perfringens* spores in ground and soil.

Although usage of anti-bacterial agents in some examined flocks, the isolation rate of *C. perfringens* was high, this result was confirmed by Ayyagari *et al.,* (2003) who found that *C. perfringens* was implicated in many cases of antibiotic associated diarrhea in rabbits.

Conclusively, *C. perfringens* enteric infection in weaned rabbits constitute a great problem that causes severe economic losses in commercial rabbitaries in Egypt.

References

Abdel-Rahman, A. A., F. A. Moustafa, and N. A. Hamd, 2006. Detection of the prevalence and pathogenicity of *Clostridium perfringens* and *Clostridium spiroforme* associated diarrhea in rabbits. Assiut Veterinary Medical Journal, 52(108):321-335.

Agnoletti, F., 2012. Update on rabbit enteric diseases: despite improved diagnostic capacity, where does disease control and prevention stand? Proceedings 10th World Rabbit Congress – September 3 - 6, Sharm El-Sheikh –Egypt, 1113- 1127.

Ayyagari, A., J. Agarwal, and A. Garg, 2003. Antibiotic associated diarrhoea: Infectious causes. Indian J Med Microbiol, 21:6-11.

Banchroft, J.D., A. Stevens, D.R. Turner, 1996. Theory and practice of histological techniques. Fourth Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo. Borriello S.P., Wilcox M.H. 1998.

Baskerville, M.; Wood, M. and J.H. Seamer, 1980. *Clostridium perfringens* type E enterotoxaemia in rabbits. Veterinary Record, 107(1):18-19.

Cocchi, M., Drigo, I., Bacchin, C., Bano, L., Marcon, B. and F. Agnoletti, 2008. Toxin-genotyping of *clostridium perfringens* strains isolated from rabbits with enteric disease. 9th World Rabbit Congress – Verona – Italy.921-924.

Cruikshank, R., Deguid, J. P., Morromain, B. P. and R. H. Swaim, 1975. Medical Microbiol. 12th ed. Vol. II, Churchil Livingstone. Edinberg, London and New York.

Diaa, H.M. 2010. Microbiological studies on *Clostridium perfringens* affecting laboratory animals. M.V.Sc. Thesis (Microbiology), Faculty of Veterinary Medicine, Cairo University.

El-Bakrey, R.M., 2009.Some studies on bacterial diseases of rabbits. Master Thesis. [Zagazig (Egypt)]: Zagazig University.

El-Deeb, M. E., 1992. Studies on the incidence of *Clostridial* organisms in domestic rabbits. M.V.Sc. Thesis (Microbiology), Faculty of Veterinary Medicine Zagazig University.

El-Rahman, M. A. and E. I. Atwa, 2006. Studies on *Clostridial* microorganisms in rabbits and the use of ELISA for detection of *Clostridium perfringens* toxins. Veterinary Medical Journal Giza, 54(3):671-684.

Finzi, A. and A. Amici, 1991. Traditional and alternative rabbit breeding systems for developing countries. Rivista di Agricultura Subtropicale e Tropicale, 6(1): 103-125.

Hunter, S. E., I. N. Clarke, D. C. Kelly and R. W. Titball, 1992. Cloning and nucleotide sequencing of the *Clostridium perfringens* Epsilon toxin gene and its expression in *E. coli*. Infectious Immunology, 60:102-110.

Ivanics, E., R. Glavits, and A. Hadhazy, 1982. Occurrence of Tyzzer's disease in brown hares (*Lepus europaeus*). Mayar Allatorvosok Lapja, 37(8):525-527.

Julian, I. Rood, 1998. Virulence genes of *Clostridium perfringens*. Annual Review of Microbiology, 52:333-360.

Khelfa, D. E. G., A. A. Wafaa and M. S. Heba, 2012. Recent Status of *Clostridial* Enteritis Affecting Early Weaned Rabbits in Egypt. Life Science Journal .9(4):2272-2279.

Koneman, E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger and W. C. Winn, 1992. Color atlas and textbook of diagnostic microbiology. J.B. Lippincott Company Philadelphia, Fourth Edition.

Kunstyr, I., I. Matthiesen, and T. Matthiesen, 1975. Acute enteritis in rabbits. Zeitschrift Versuch, 17(1):57-63.

Lebdah, M.A. and A.M. Shahin, 2011. Clostridia as an etiological agent of mucoid enteropathy in rabbits. Nat Sci. 9:63-72.

Lee, W. K., T. Fujisawa, S. Kawamura, K. Itoh, and T. Mitsuoka, 1991. Isolation and identification of *Clostridia* from the intestine of laboratory animals. Laboratory Animal; 25(1):9-15.

Macfaddin, J. F., 2000. Biochemical test for identification of medical bacteria. 3rd Ed. Lippin Cott Willians and Willions, Washingtion, Philadelphia, USA.

Mariano, E. F., J. F. Derek, P. Rachael, S. Sameera, A. Vicki, I. R. Julian, A. M. Bruce, and A. U. Francisco, 2007. Both epsilon-toxin and beta-toxin are important for the lethal properties of *Clostridium perfringens* Type B isolates in the mouse intravenous injection model. Infection and Immunity, 75(3):1443-1452.

McDonel, J. L. and C. L. Duncan, 1975. Histopathological effects of *C. perfringens* enterotoxin in rabbit ileum. Infectious Immunology, 12 (5):1214-1218.

Nagahama, M., S. Ochi, M. Oda, K. Miyamoto, M. Takehara and K. Kobayashi, 2015. Recent Insights into Clostridium perfringens Beta-Toxin. Toxins, (7): 396-406.

Nashwa, A. E., M. E. Mamdouh, M. D. Heba, M. S. Eman, and A. A. Mohamed, 2014. Genotyping Characterization on Clostridium Perfringens Affecting Laboratory Animals. Advances in Environmental Biology, 8(5): 1480-1492.

Nagi, G. M., A. Laila, M. H. Ebeid, and M. El-Sagheer, 1988. Afield study on role of *Clostridium perfringens* in rabbit's diarrhea complex. Veterinary Medical journal, 36(2): 221-230.

Percy, D.H., C.A. Muckle, R.J. Hampson, and M.L. Brash, 1993. The enteritis complex in domestic rabbits: A field study. Can. Vet. J. 34: 95-102.

Petit, L., M. Gilbert, and M. R. Popoff, 1999. *Clostridium perfringens:* toxinotype and genotype. Trends Microbiology, 7:104-110.

Prescott, J. F., 1977. Tyzzer's disease in rabbit in Britain. Vet. Rec., 100(14):285-286.

Saggiorato M., G. Pradella, S. Scandurra, C. Bacchin, T. Ferro, F. Agnoletti, 2008. Tylosin Mic Distribution from Clinical Isolates of Clostridium Perfringens in France, Italy and Spain. 9th World Rabbit Congress .Verona .Italy.

Scharmann, W. and D. Wolff, 1985. Occurrence and prevention of Tyzzer's disease in rabbit colony. The contribution of laboratory animal science to the welfare of man and animal, 8th ICLAS/CALAS symposium, Vancouver, 53-57.

Shi XiShan, Wang CunLian and Xu Tong, 2008. Diagnosis and treatment of *Clostridium welchii* in Rex rabbit. Chinese Journal of Rabbit Farming, 3: 36-37.

Smith, L. D. and L. V. Holdman, 1968. The pathogenic anaerobic bacteria. Charleshomas publisher, USA. 1st ed., 201-255.

Sting, R., 2009. Detection of beta2 and major toxin genes by PCR in *Clostridium perfringens* field isolates of domestic animals suffering from enteritis or enterotoxaemia. Berliner und Munchener Tierarztliche Wochenschrift. (122) 9/10: 341-347.

Szemeredi, G. Palfi and I. Gaco, 1983. Etiology of diarrhea in rabbits at weaning. Magyar Allatrovosok Lapja, 83(5): 280-283.

Uzal, F. A., W. R. Kelly, R. Thomas M. Hornitzky and F. Galea, 2003. Comparison of four techniques for the detection of *Clostridium perfringens* type D epsilon toxin in intestinal contents and other body fluids of sheep and goats. Journal of Veterinary Diagnostic Investigation. 15(2): 94-99.

Vaikosen, E.S. and W. Muller, 2001. Evaluating biochemical tests for isolation / identification of *C. perfringens* in fecal samples of small ruminants in Nigeria. Bulletin of Animal Health and Production in Africa, 49 (4): 244- 248.

Wilber, J.L. 1999. Pathology of the rabbit. Department of veterinary pathology, Armed Forces Institute of Pathology. Washington, D.C. pp: 10-19.

Youhanna, S. S. and J. Glenn Songer, 2006. Clostridium perfringens: Insight into virulence evolution and population structure. Anaerobe 12 (2006) 23–43.