



## WEANLING RABBIT MORTALITIES CAUSED BY ENTEROPATHOGENIC BACTERIA: BACTERIOLOGICAL AND PATHOLOGICAL INVESTIGATION

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### ABSTRACT

Samples of internal organs (liver, heart, spleen, kidney and intestinal contents) were aseptically collected from 120 freshly died newly weanling rabbits and subjected to isolation and identification of the causative bacterial pathogens. The causative pathogens were isolated and identified biochemically. *E.coli* and *Salmonella* (the major associated pathogens) were typed serologically and tested for antimicrobial agents. The bacterial infection prevalence rate was *Escherichia coli* (56.6%), *Salmonella* spp. (27.5%), *Enterobacter* spp. (7.5%), *Citrobacter* spp. (5%) and *Proteus* spp. (3.3%). Out of the 68 infections with *E.coli*, 30 were serotyped as O<sub>125</sub> (ten), O<sub>127</sub> (six), O<sub>128</sub> (five), O<sub>86</sub> (five) and untyped (four). Out of the 33 *Salmonella* infections, seven were serotyped as serovar *S. goldcoast* (four) and serovars *S. magherafelt* (three). *E.coli* serogroups were resistant to the majority of used antimicrobial and were sensitive only to Sulphamethazole. Both *Salmonella* serovars were sensitive to most antimicrobial used in this study but they were resistant to amoxicillin. Both infected rabbit groups with *E.coli* and *Salmonella* demonstrated obvious histopathological alterations in the intestine, liver and spleen. Both *E.coli* (O<sub>86</sub>) and *Salmonella* goldcoast were used for experimental infection of weanling rabbits (6-8 weeks). Five days post-infection and after observation of the clinical symptoms, animals were sacrificed and tissue samples from the intestine, liver, kidney and spleen were examined histopathologically. Utmost care must be taken around the time of weaning in rabbits.

**Keywords:** Antibiogram, *Citrobacter* spp, *E.coli*, Mortality, Rabbit.

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### INTRODUCTION

Age of weaning (6-8 weeks) has a bearing on growth, production and survival of kits (**Obike et al., 2014**). Weaning age is one of the critical components under management practices which can affect the profitability of a rabbit enterprise, because of its consequence on productive traits, mortality, carcass and meat quality properties in rabbits (**Assan, 2017**).

In the last few years there has been a growing interest in understanding the causes of deaths in weanling rabbits. In this context, enteropathy has been always considered as a major problem in rabbit breeding and has been reported to cause high losses (about 30% from birth to slaughter) in France, as shown in the production records of around 850 rabbit farms (**Guerder, 2001**). Additionally, enteritis has been recorded as

the main cause of morbidity and mortality in rabbits, which was caused by various pathogenic agents. Pathogens causing enteritis are transmitted by the fecal-oral route and the infection, where one of the main predisposing factors is the unbalanced diet. (**Newton et al., 2004**) reported that *E. coli* was the predominant bacteria isolated from colon and caecum of diarrhetic rabbits. This impairment seems to be responsible for the intensive colonization of the intestinal tract by various microorganisms with pathogenic potential, whose effects manifest as diarrhetic syndrome (**Petrov et al., 2005**). **Moura et al., (2009)** stated that colibacillosis represents a major cause of diarrhea especially in young rabbits.

Among pathogens causing enteritis, some strains of *Clostridium* species, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* species, *Coccidia* and *Salmonella* species were widely

involved in enteric infections of young rabbits (**Eid and Ibraheem 2006**).

**Croxen and Finlay (2010)** considered *Escherichia coli* a remarkable and diverse bacterium. This normally harmless commensal needs only to acquire a combination of mobile genetic elements to become a highly adapted pathogen capable of causing a range of diseases. In rabbit *Escherichia coli* enteritis is frequent, especially when it involves the attaching and effacing enteropathogenic *E.coli* (AEEC), which are now considered to be an important cause of diarrhea in suckling and weanling rabbits (**Camguilhem et al., 1986; Okerman, 1987**). Mortality varies from very low to very high, according to the strains involved. As seen by electron microscopy, these strains have the ability to heavily colonize the distal Regions of the intestinal tract, with typical cuplike adhesion to enterocytes in vivo (**Federighi et al., 1989**).

The involvement of *Salmonella* species in rabbit enteritis is controversy. Some reports mentioned that it is uncommon and infections range from asymptomatic carriage and localized gastroenteritis to systemic enteric fevers (**Bradley et al., 1994**). However, others mentioned that *Salmonella* causes high mortality rate that may reach 80% in rabbits at 4-8 weeks old (**Saif-Eldin et al., 1994**). In addition, salmonellosis also has been reported to induce high morbidity and mortality rates in rabbit farms, especially in young rabbits (**Borrelli et al., 2011**).

Different *Salmonellae* including *S. Enteritidis*, *S. Mbadaka*, *S. Sheidelburg* *S. Typhimurium* and *S. Pullorum* were isolated from rabbits at weaning time (**Saad, 1970**). Moreover, *S. Newport* was recorded for the first time from rabbits in Egypt by **Abd El Gwad (1988)**. **Abd El Gwad (1988) and Abd El Azeem (1995)** reported that *S. Typhimurium* represented a high percentage of bacteriological causes infecting young rabbits (1-4 weeks) with a significant bad impact on production of rabbit's farms. In addition, **Ibraheem (1999)** isolated *S. Arizona*, *S. Typhimurium* and *S. Dublin* from rabbits suffering from enteritis. The current study aims to precisely verify the actual role of *E.coli* and *Salmonella* in enteric infections that cause losses in weanling rabbits, detection of other pathogens causing mortalities and detect the most frequent isolates and use drug of choice according to sensitivity test for treatment.

## MATERIALS AND METHODS

### Samples and cultivation

Samples of internal organs (liver, heart, spleen, kidney and intestinal contents) were

aseptically collected from 120 freshly died newly born rabbits in Assuit governorate from different localities. The samples were enriched in buffer peptone, then directly inoculated onto MacConkey's agar and sub cultured onto Eosin Methylene Blue Agar (EMB). The inoculated plates were incubated at 37°C for 24- 48 hours. The isolation, purification and biochemical identification of the bacterial isolates were carried out as per standard protocols (**Koneman et al., 1997**). Also, 1 ml of buffer peptone was inoculated into Tetrathionate broth with Neomycin and iodine and incubated at 37° C for 24 hours, then sub cultured onto Xylose Lysine Deoxycholate (XLD) agar and inoculated plates were incubated for 24 hours at 37°C. The suspected colonies were identified morphologically and biochemically according to international standards (**ISO 6579, 2017**) for isolation of *Salmonella*.

### Serological identification

Antisera of *Escherichia coli* were used for serotyping of somatic "O" antigen according to **Ewing et al. (1986)** using slide agglutination test (polyvalent sera, 8 vials; monovalent sera, 43 vials). Antisera were obtained from DENKA SEIKEN CO. LTD Tokyo, Japan.

Serotyping of suspected *Salmonella* isolates was performed according to WHO collaborating Center for reference and Research on *Salmonella* antigenic formulae of *Salmonella* serovars according to **White Kauffmann Leminor Scheme (Grimont and Weill, 2007)**. Antisera of *Salmonella* were obtained from MAST ASSURE™ SALMONELLA ANTISERA England.

### Antibiogram pattern

Antibiotic susceptibility test was performed by disc diffusion method according to Clinical Laboratory Standard Institute (**CLSI; M100-S21, 2011**) to different "O" serogroups of *E.coli* and *Salmonella* serovars recovered from newly born rabbits. The used antibiotics included amoxycillin, Cefepime, Cephalothin, Levofloxacin, Ceftriaxone, Cefotaxime, Ceftazidime, Norfloxacin, Oxytetracycline, Sulphamethazole and Gentamicin (Oxoid) for susceptibility testing. Zones of inhibition were measured after 24-hour incubation at 37°C. Interpretation of sensitivity and resistance was based on guidelines of European Committee on Antimicrobial Susceptibility Testing (**EUCAST, 2016**).

### Experimental animals

Eighteen New Zealand White rabbits (6-8 weeks old) were used, their feces were free from *Eimeria* oocysts, *E. coli* and *Salmonella* as proved

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by parasitological and bacteriological examination. They were housed in metal cages each of three animals at room temperature (21-23°C) and were watered and fed ad libitum with a commercial pellet and green food (alpha alpha) without coccidiostatic throughout the experiment.

### Experimental design

Eighteen rabbits were allocated into three groups, 6 rabbits of **group (1)** served as control group (Negative Control) were orally inoculated with the saline only. Rabbits of **group (2)** were orally inoculated with the  $0.5 \times 10^6$  Colony forming Unit (CFU) of the isolated *E.coli* serotypes ( $O_{86}$ ), rabbits of **group (3)** were orally inoculated with  $0.5 \times 10^8$  CFU of the isolated *Salmonella* Goldcoast (Onyekaba,1985). The animals were observed twice a day for at least 10 minutes. The clinical signs such as abdominal distension, diarrhea and anorexia were checked on a daily basis. All rabbits were sacrificed at 5<sup>th</sup> day post infection (DPI) and a complete post-mortem examination was performed on all rabbits. Animals of group 2 and 3 were proved for the presence of *E.coli* and *Salmonella* in their caecal contents by isolation and biochemical identification, respectively as mentioned above. All care and experimental procedures were approved by the animal care and ethics committee of the Faculty of Veterinary Medicine, Assiut University.

### Histopathological examination

Immediately after the animals were sacrificed, tissue specimens (one cubic centimeter) were taken from liver, kidney, spleen and intestine at 5<sup>th</sup> day post infection from all experimental

groups, fixed in 10% buffered formalin, dehydrated in ascending concentrations of ethanol, cleared in xylene and embedded in paraffin. Thin sections (4µm thick) were cut by microtome and stained with Hematoxylin and Eosin stain (H&E) and examined microscopically (**Bancroft and Gambl, 2008**).

### Transmission Electron Microscopy

Small tissue blocks (one cubic millimeter) were taken from fresh ileum and colon of *E.coli* and *Salmonella* infected rabbits, fixed in 2% buffered glutaraldehyde for 2 hr., osmicated in 1% osmium tetra oxide then dehydrated in graded ethanol alcohol and embedded in Epon 812. Semi thin sections were cut and stained with 0.5% Toluidine blue for orientation and selection (**Bancroft and Gambl, 2008**). Ultrathin sections were stained with uranyl acetate and lead citrate, and examined by Jeol, CX11 electron microscope in Electron Microscope unit, Assiut University.

## RESULTS

### Bacteriological examination

In this study, the bacteriological examination of internal organs (liver, heart, spleen, kidney and intestinal contents) from 120 freshly dead weanling rabbits was carried out and showed that the bacterial infection prevalence rate was *Escherichia coli* (56.6%), *Salmonella* spp. (27.5%), *Enterobacter* spp. (7.5%), *Citrobacter* spp. (5%) and *Proteus* spp. (3.3% (Table 1). The biochemical tests are shown in Table (2).

Table 1: Prevalence rate of pathogenic strains of *E.coli*, *Salmonella*, *Citrobacter*, *Enterobacter* and *Proteus* isolates among internal organs (liver, heart, spleen, kidney and intestinal contents) from examined freshly dead newly born rabbits samples.

Total Number of examined samples	<i>E. coli</i>		<i>Salmonella</i>		<i>Citrobacter</i>		<i>Enterobacter</i>		<i>Proteus</i>	
	No.	%*	No.	%*	No.	%*	No.	%*	No.	%*
120	68	56.6	33	27.5	6	5	9	7.5	4	3.3

\* The percentage of each isolate according to total Number of examined samples.

Table 2: Biochemical characterization of isolated pathogens.

Test	Catalase	Oxidase	Methyl red	Vogus proskaures	Indole	Citrate	Urease	Triple sugar iron	H2S	Motility
<i>E.coli</i>	+	+	+	-	+	-	-	A/A	-	+
<i>Salmonella</i>	+	-	+	-	-	-	-	AK/A	+	+
<i>Enterobacter</i>	+	-	-	+	-	+	+/-	A/A	-	+
<i>Citrobacter</i>	+	+	-	+	+	+	-	A/A -A/K-A	+	+
<i>Proteus</i>	+	+	+	-	-	+/-	+	AK/A	+	+

**Serological identification**

The isolated *E.coli* and *Salmonella* spp. were serotyped and revealed that, out of the 68 infections with *E.coli*, 30 were serotyped as O<sub>125</sub> (ten), O<sub>127</sub> (six), O<sub>128</sub> (five), O<sub>86</sub> (five) and untyped (four). Out of the 33 *Salmonella* infections, seven were serotyped as serovar *S. goldcoast* (four) and serovars *S. magherafelt* (three) as shown in Table (3).

Table 3: Serological identification of pathogenic strain of *E.coli* strains and *Salmonella* serovars isolated from freshly dead newly born rabbits.

<i>E.coli</i> strains (30)*			<i>Salmonella</i> Serovars(7)*		
Serogroups	Number	Percentage**	Serotypes	Number	Percentage**
O <sub>125</sub>	10	33.33%	<i>S. Goldcoast</i>	4	40%
O <sub>127</sub>	6	20%	<i>S. Magherafelt</i>	3	30%
O <sub>128</sub>	5	16.66%			
O <sub>86</sub>	5	16.66%			
Untyped	4	13.33%			

\* The total number of serotyped isolates.

\*\*The percentages were calculated according to the total number of serotyped isolates.

**Antimicrobial sensitivity:**

By comparing Antimicrobial susceptibility tests, the majority of the tested isolates of *E.coli* serogroups were resistant to Amoxicillin, cefepime, cephalixin, oxytetracycline, levofloxacin, Ceftriaxone, Cefotaxime and Ceftazidime. Most *E.coli* isolates were sensitive to Sulphamethazole. On the other hand, *S.goldcoast* was sensitive to norfloxacin, ceftriaxone and cefepime. However, *S.magherafelt* was sensitive to norfloxacin, sulphamethazole, ceftazidime ceftriaxone and levofloxacin (table 4).

Table 4: Antimicrobial sensitivity test of *E.coli* isolates and *Salmonella* serovars recovered from freshly dead newly born rabbits.

<i>Chemotherapeutic agent</i>	<i>E.coli</i> Serotypes								<i>Salmonella</i> Serovars			
	O <sub>125</sub> (4)		O <sub>127</sub> (7)		O <sub>128</sub> (3)		O <sub>86</sub> (8)		S. Gold coast (4)		S. Mag. (3)	
	S		S		S		S		S		S	
	No	%	No	%	No	%	No	%	No	%	No	%
Amoxycillin	2	50	3	42.88	3	33.33	6	75	1	25	1	33.3
Cefepime	1	25	3	42.88	2	66.66	5	62.5	3	75	1	33.3
Cephalixin	2	50	3	42.88	2	66.66	7	86.25	2	50	1	33.3
Levofloxacin	3	75	2	25.71	1	33.33	3	37.5	2	50	2	66.66
Ceftriaxone	2	50	3	42.88	2	66.66	4	50	3	75	2	66.66
Cefotaxime	2	50	3	42.88	2	66.66	5	62.5	2	50	1	33.3
Ceftazidime	1	25	2	25.71	1	33.33	4	50	2	50	2	66.66
Norfloxacin	1	25	4	100	2	66.66	5	62.5	4	100	3	100
Oxytetracycline	0	0	0	0	0	0	0	0	0	0	0	0
Sulphamethazole	4	100	4	100	3	100	8	100	2	50	3	100
Gentamicin	3	75	2	50	1	33	-	-	2	50	1	33.3



### Clinical signs and postmortem findings of experimentally infected rabbits

The clinical signs included mild watery diarrhea and abdominal distension in both *E.coli* and *Salmonella* inoculated rabbits. *E.coli* O<sub>86</sub> induced the most severe clinical symptoms among inoculated rabbits, however, *Salmonella* goldcoast induced mild symptoms. Postmortem findings revealed congested intestine with signs of enteritis. The intestine was also distended and filled by clear to yellow watery contents with variable amounts of mucus and gases in some rabbits. Other organs (liver, kidney and spleen) showed mild degree of congestion and enlargement. The severity of gross lesions was variable in all rabbits of both groups. Uninoculated rabbits showed no clinical signs or gross lesions in the above-mentioned organs.

Histopathologically, both inoculated groups with *E.coli* and *Salmonella* showed focal areas of necrosis in the liver. A mononuclear cellular infiltration was demonstrated in the liver of *E.coli* inoculated group (Fig. 1-A), dilatation of central vein and blood sinusoids, necrobiotic changes of hepatocytes and focal infiltration of polymorph nuclear leucocytes in the liver of *Salmonella* infected rabbit (Fig. 1-E). In the kidney, glomerular swelling and necrobiotic changes in renal tubules in the kidney of *E.coli* infected rabbit (Fig. 1-B), glomerular swelling with increased mesangial cells, congested blood vessel and necrobiotic changes of renal tubular epithelium in the *Salmonella* infected rabbit (Fig. 1-F). The spleen showed lymphocytic depletion in some splenic corpuscles, congestion and hemorrhages in the spleen of *E.coli* infected rabbit (Fig. 1-C); slight lymphocytic depletion in some splenic corpuscle with mild congestion and hemorrhages in *Salmonella* infected rabbit (Fig. 1-G). The rabbit groups inoculated with both *E. coli* and *Salmonella* demonstrated damaged and swollen

enterocytes at the villi tips and the villi are blunted with edema and heterophilic infiltration of the lamina propria in both small (jejunum) and large intestine (colon) (Figs. 1-D& H).

In semi-thin sections, of rabbits inoculated with both *E.coli* and *Salmonella* showed degenerative changes of ileal mucosa manifested by focal necrosis of epithelial cells (Fig. 2-A). Crypt cells demonstrated hyperplasia with various cytoplasmic inclusions, congested blood vessel in submucosa (Fig. 2-B). The strains of *Salmonella*, in particular, caused ballooning dilation of epithelial cells, accumulation of intracytoplasmic debris and disappearance of the brush border of swollen cell (Fig. 2-C). In addition, intraepithelial bacteria were seen in the crypt epithelial cells with hemorrhages in the submucosa (Fig. 2-D). Using transmission electron microscope, the intestine of rabbits inoculated with strains of *E.coli* showed that microvilli are irregular, short and blunt, bacteria and debris were visualized in intestinal lumen. The cytoplasmic organelles were well preserved (Fig. 3-A). Many fat droplets were present in the cytoplasm of the enterocytes (Fig. 3-B). Heterophil cell and macrophage containing engulfed organisms were encountered in the lamina propria beneath epithelial cells (Fig. 3-C). The intestine of rabbits inoculated with *Salmonella* spp. showed that microvilli of crypt cells were almost obliterated the lumen, they appeared irregular, short, and blunt. The enteric cells also showed accumulation of intracytoplasmic dense granules and swollen endoplasmic reticulum while the goblet cells were abundant (Fig. 3-D). In addition, various cytoplasmic inclusions, double-membrane bound cytoplasmic dense bodies, swollen and dilated endoplasmic reticulum were also demonstrated in the enteric cells (Fig. 3-E& F).

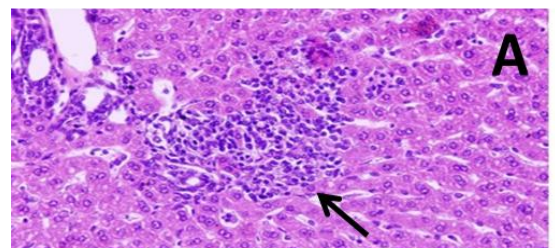


Fig. 1: Histopathological paraffin sections of liver (A and E), kidney (B and F), spleen (C and G) and intestine (D and H) of rabbits inoculated with *E. coli* and *Salmonella* spp.; respectively. The liver of *E. coli* infected rabbit (A) demonstrates focal necrosis with mononuclear cellular infiltration, however that of *Salmonella* inoculated rabbits (E) demonstrates dilatation of central vein and blood sinusoids, necrobiotic changes of hepatocytes and focal infiltration of polymorph nuclear leucocytes (arrow). In the kidney, *E. coli* infected animals (B) shows glomerular swelling (arrow) and necrobiotic changes in renal tubules (arrowhead), whereas those inoculated with *Salmonella* spp. (F) displayed glomerular swelling with increased mesangial cells (arrow) congested blood vessel (arrowhead) and necrobiotic changes of renal tubular epithelium. In the spleen, *E. coli* inoculation (C) caused lymphocytic depletion of splenic corpuscle (arrow), splenic congestion and hemorrhages (arrow head), but *Salmonella* inoculation (G) induced slight lymphocytic depletion of splenic corpuscle (arrow), splenic mild congestion and hemorrhages (arrow heads). The enterocytes at the villi tips are damaged and swollen (arrows) and the villi are blunted with edema and heterophilic infiltration of the lamina propria in the intestine of *E. coli* infected rabbit (D), however in *Salmonella* inoculated rabbits (H) there is degeneration of enterocytes at the villi tips (arrows) and heterophilic infiltration of the lamina propria. Stain: Hematoxylin and Eosin (X 200).

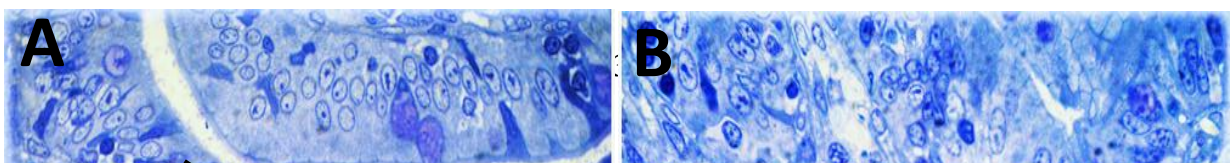


Fig. 2: Semi-thin section micrograph of the ileum of rabbits inoculated with *E.coli* (**A and B**) and *Salmonella* spp. In *E.coli* inoculated animals, there is degenerative changes of ileal mucosa manifested by focal necrosis (arrows) of epithelial cells (**A**), crypt cells hyperplasia with various cytoplasmic inclusions (white arrows) and congested blood vessel (black arrows) in submucosa (**B**). Ballooning dilation of epithelial cells, accumulation of intracytoplasmic debris (arrowhead) and distortion of the brush border of swollen cell (**C**), Intraepithelial bacteria (white arrow) in crypt epithelial cells and hemorrhage (black arrow) in submucosa (**D**). Toluidine blue stain, (x1000) bar = 20µm.



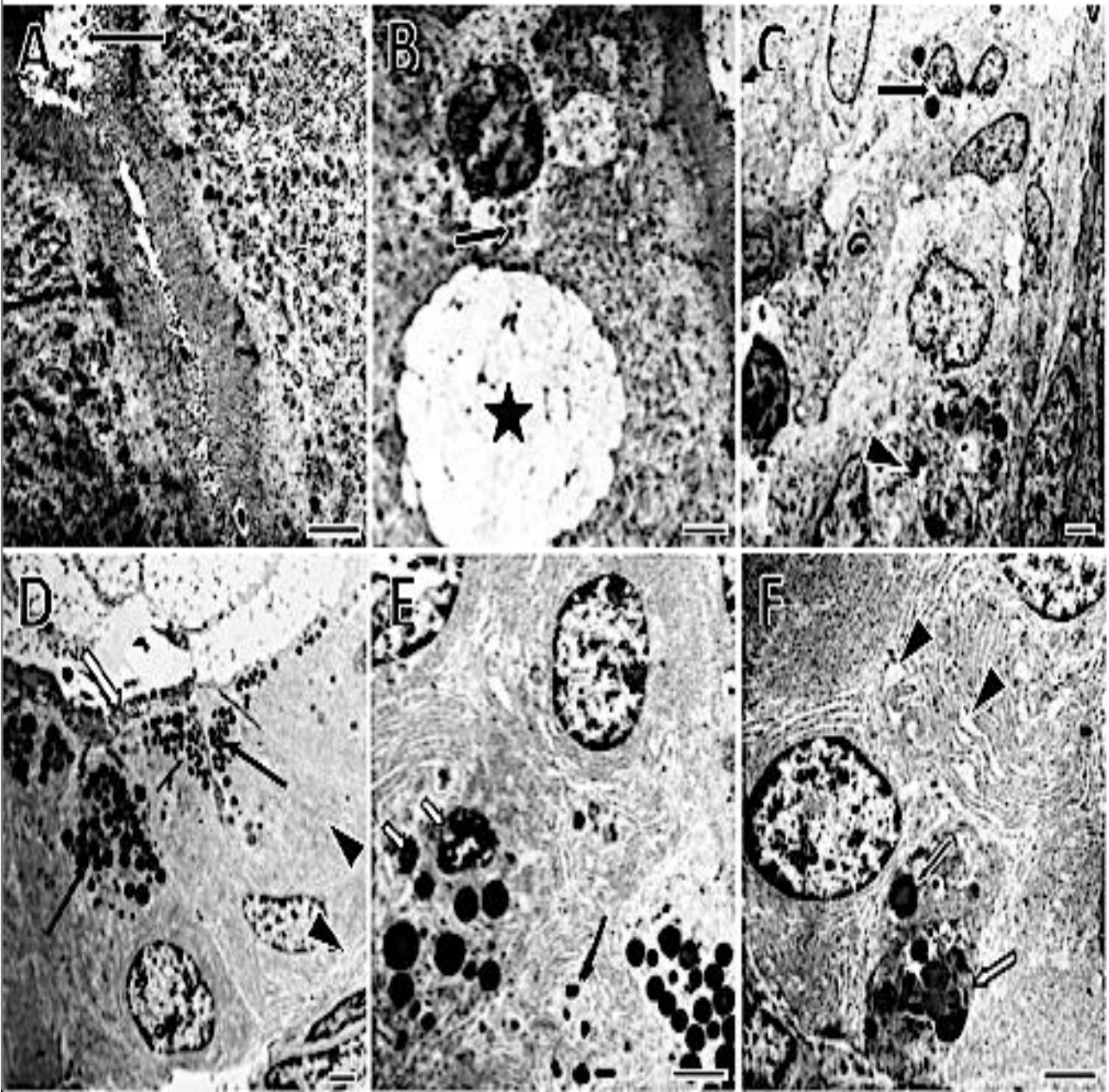


Fig. 3: Transmission electron micrograph of sections from the ileum of rabbits inoculated with *E.coli* (A, B, C) and *Salmonella* spp.(D, E, F), bar = 2 $\mu$ m. Panel (A) shows two apposed epithelial lining of 2 villi with bacteria and debris are in intestinal lumen (arrows), note that cytoplasmic organelles were well preserved, (x5800). Panel (B) demonstrates many fat droplets in the cytoplasm of the enterocyte (arrow) and adjacent goblet cell full of secretions (asterisk) (x4800). Panel (C) shows Heterophil cell (arrow) and macrophage containing engulfed organisms (arrow head) in the lamina propria beneath epithelial cells (x2900). Panel (D) displays accumulation of intra cytoplasmic dense granules (thin arrows) swollen endoplasmic reticulum (arrowheads) with irregular, short and blunt microvilli (white arrow), notice abundant goblet cells (x2900). Panel (E) demonstrates epithelial cells cytoplasmic inclusions (white arrows), double-membrane bound cytoplasmic dense bodies (thin black arrows) and swollen endoplasmic reticulum (head arrow) (x5800). Panel (F) shows intracytoplasmic membrane-bound inclusions (arrows) and dilated endoplasmic reticulum (head arrows) (x5800).

## DISCUSSION

This paper is a modest contribution to the ongoing discussion about the causes of the great losses of weanling kits in rabbitries. The data obtained were broadly consistent with the major trends in this issue. The current study, showed the recovery rate for *E. coli* (56.6%) and *Salmonella* (27.5%). Only 15.8% of these samples showed infections with other bacterial species including *Citrobacter* (5%), *Enterobacter* (7.5%) and *Proteus* (3.3%) from internal organs (liver, heart, spleen, kidney and intestinal contents) which collected and pooled from the freshly died weanling rabbits. These current findings were agree with previous studies which emphasized that *E. coli* was the most prevalent pathogen causing diarrhea, enteritis and mortalities in young and weanling rabbits (Otaru *et al.*, 1990; Milon *et al.*, 1999; Taddei *et al.*, 2005; Mohamed *et al.* 2013). The involvement of other pathogens, other than *E. coli* and *Salmonella*, in intestinal disorders of rabbits has also been recorded (Eid and Ibraheem, 2006).

The finding of *E. coli* Serotyping demonstrated that they included O<sub>125</sub>, O<sub>127</sub>, O<sub>128</sub> and O<sub>86</sub>. Previous studies verified the presence of many serotypes of *E. coli* that were isolated from rabbits suffering from intestinal disorders in Egypt, which included serotypes O<sub>55</sub>, O<sub>119</sub>, O<sub>126</sub>, O<sub>128</sub>, O<sub>78</sub>, O<sub>44</sub>, O<sub>111</sub>, O<sub>114</sub>, O<sub>26</sub>, O<sub>75</sub>, O<sub>103</sub>, O<sub>145</sub> and O<sub>158</sub> (Abd El Azeem, 1995; Abbas 2002; El Bakrey 2009; Hassan and Abd Al Azeem 2009; Shahin *et al.*, 2011). It has been also mentioned that serotypes O<sub>55</sub> and O<sub>126</sub> were highly pathogenic, while serotypes O<sub>119</sub> and O<sub>128</sub> were non or less pathogenic (Saad, 1970; Bekheet, 1983).

The recent findings partly were agree the previous reports that O<sub>128</sub> was the least pathogenic strain as proved by the mild symptoms caused by this strain in the current experimental infection. Based on the current findings, The majority of the isolated *E. coli* serotypes were resistant to most of the tested antimicrobial and were sensitive only to Sulphamethazole. In the same respect, Kylie *et al.*, (2017) mentioned that at least one *E. coli* isolate was resistant to at least one antimicrobial agent in samples from 55.6% of commercial rabbit farms. In addition, the resistance of *E. coli* isolated from 32 Italian industrial rabbit holdings to a wide variety of antimicrobial agents like tetracyclines, trimethoprim, enrofloxacin, chloramphenicol and colistin has been reported by Agnoletti *et al.*, (2018). The last authors added that the colistin resistance gene *mcr-1* was found to be widely present in rabbit farming. On the other hand,

both isolated *Salmonella* serovars were sensitive to most of the tested antimicrobial in this study but they were resistant to amoxicillin. Similarly, Mijovic *et al.* (2012) verified the sensitivity of *Salmonella* spp. from human stools to a wide range of antibiotics although they recorded their resistance to ampicillin.

The rabbit groups inoculated with *E. coli* and *Salmonella*, in the current study, showed diarrhea, enteritis accompanied by intestinal gas distention and watery contents, in addition, the internal organs showed mild degree of congestion and enlargement. In this context, Joseph (1993), Christian and Chris (2002) believed that the manifestation of septic shock in form of severe congestion, edema, hemorrhage, and Heterophils infiltrates in the internal organs of mice occur as a result of the lipopolysaccharides which are major constituents of gram-negative bacteria and that lead to diarrhea and enteritis in mice. On the other hand, invasive organisms, as exemplified by *Shigella*, *Salmonella*, and some *E. coli* strains, provoke diarrhea by invading and multiplying within the intestinal mucosa, causing mucosal damage and fluid loss (Giannella *et al.*, 1973).

*Klebsiella* spp., *Enterobacter*, *Pseudomonas* spp. *Proteus* spp. were represent other member of gram negative bacilli isolated from intestinal content of rabbits with intestinal disorders (Mostafa *et al.*, 1993). Eraqi (2006) reported that the most important bacterial pathogens having a significant role in causing diarrhea in rabbits was *Enterobacteriaceae*, which including *E. coli*, *Pseudomonas* spp. *Proteus* spp., *Klebsiella* spp., *Citrobacter* spp. and *Salmonella* spp. He also isolate O<sub>128</sub>, O<sub>111</sub>, O<sub>26</sub>, O<sub>86</sub>, O<sub>127</sub>, O<sub>78</sub>, O<sub>126</sub>, O<sub>27</sub>, O<sub>119</sub>, O<sub>153</sub>, O<sub>20</sub>, O<sub>125</sub> and O<sub>1</sub> serogrouping of *E. coli*. and S. Typhi, S. Minnesota and S. Thompson. Also this study revealed high sensitivity of isolated *E. coli* strains to nalidixic acid, pefloxacin, flucloxacillin, cefoperazone and amoxicillin+clavulonic. While isolated *Salmonella* spp. Showed highly sensitivity to gentamycin, sulbactam+ampicillin and flucloxacillin. In contrast with the current results, Camarda *et al.*, (2013) declared that S. Typhimurium is a common serovar causing abortion or systemic infection in rabbits at different stages, especially before and during weaning.

Upon histopathological examination, both *E. coli* and *Salmonella* inoculations induced pathological changes in the liver, kidneys, spleen and intestine of experimental rabbits. Lesions caused by *E. coli* in the liver and kidneys had been recorded consistently by Keenan, *et al.*, (1986),

Padhye *et al.*, (1987) and Alaa, *et al.*, (2018). Our results were agreed with the above-mentioned authors in that the lesions mostly included necrosis of the hepatocytes with mononuclear cellular infiltration in the liver and hydropic degeneration of renal tubules, proliferation of mesangial cells and congestion of blood vessels in the kidney. The spleen, however, demonstrated congestion and hemorrhages in red pulp and lymphocytic depletion in white pulp. Whereas Riley (1987), observed proliferative changes in the spleen that included both white and red bulbs in addition to amyloid degeneration and focal necrosis in the spleen of rabbits. Comparatively, the *Salmonella* inoculated rabbits showed less severe pathological changes in liver, kidneys and spleen than those observed in *E.coli* inoculated animals.

Indeed, the intestinal histopathological alterations were characteristic in both *E.coli* and *Salmonella* inoculated rabbits. The intestinal lesions associating *E.coli* infection, in the current work, concur broadly with those described by (Coussement *et al.*, 1984) who observed many bacteria adhered to the luminal side of enterocytes of crypts and villi, desquamation of enterocytes, villous atrophy and inflammation of the lamina propria in the ileum, caecum and colon of experimentally infected one day old rabbits inoculated with a suspension of an *E. coli*. Similarly, Dewrée *et al.*, (2007) observed bacteria with bacillus morphology attached at sites of serious cell damage, which were demonstrated intraepithelial or in the intercellular space.

The intestinal lesions caused by *Salmonella* inoculation in the current investigation were in general agreement with the early results of Takeuchi and Sprinz (1967) who observed epithelial changes ranging from derangement of the endoplasmic reticulum to severe degenerative alterations, in addition to presence of intra-epithelial bacteria in the gut mucosa of salmonella infected guinea pig. However, we couldn't identify any intraepithelial bacilli in the current study. In the same context, Bradley *et al.*, (1994) concluded that *S.Typhimurium* was cytotoxic for the M cells (specialized epithelial cells that are found exclusively in lymphoid follicle associated epithelium, FAE). The latter authors added that destruction of an M cell creates a gap in the FAE which allow organisms to invade enterocytes adjacent to the dead cell.

## CONCLUSIONS

*E. coli* and *Salmonella* were the most commonly encountered bacterial pathogens

associated with mortalities in weanling rabbits. While *E.coli* isolates demonstrated resistance to a wide range of antimicrobials, both *Salmonella* serovars were sensitive to almost all tested antibiotics. Utmost care must be taken around the time of weaning in rabbitries. Cleaning and sanitation programs and personal hygiene very important and should be occurred in farmer houses and poultry shops to avoid contamination, minimize infection and mortality rate.

## Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript.

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