

Bacteriological Studies on *Escherichia Coli* Infection in Rabbits

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Abstract

A total number of 125 diseased rabbits collected from different farms in Port said Governorate during the period from January 2016 till March 2017 showing clinical signs of colisepticaemia ,with average age of 1-2 month were examined, 625 collected samples from liver, Intestine ,spleen, kidney and Heart blood (30.4%,29%,8.7%,17.4% and14.5%) were positive for *E.coli* respectively. These isolates were further characterized by polymerase chain reaction. *E.coli* was isolated in 525 of the 625samples (84%). The isolated *E.coli* strain were found to belong to O stereotypes in order of frequency O 153, O 125, O 27,O158 and Untypable (28%,16%,24%,12% and 20) isolates respectively. Based on PCR, All examined *E.coli* were positive100 %(5/5) for *phoA* virulence gene, while 60 %(3/5) of the tested *E.coli* isolates were positive to *eaeA* gene while 20 %(1/5) of the tested *E.coli* isolates were positive to *Tsh* gene,while all tested isolates gave negative electrophoresis of Shiga-Like Toxin gene (*stx1 and stx2*).

Introduction

Rabbit is one of the best white meats available on the market today because it has high percentage of easily digestible protein. Rabbits are easy to raise and can be produced by all strata of our society under good management and using modern farming techniques.*E. coli* is usually present in the alimentary tract of healthy rabbits, and does not normally cause diarrhea. However, enteropathogenic strains can be transferred from the doe to her kits through fecal contact (*Okerman, 1994*). Certain serotypes of this bacterium have acquired some

virulence-associated genes that enable them to cause intestinal or extra-intestinal disease. Those serotypes that cause enteric infections are generally called diarrheagenic *E. coli* strains, and their pathogenesis is associated with a number of virulence attributes, which vary according to pathotype (*Xia et al., 2010*). Enteropathogenic *E. coli* (EPEC) is the only known class of *E. coli* in rabbits which induces acute intestinal pathology marked by inflammatory lesions of the gut where these *E. coli* are strictly located (*Licois, 2004*). Recent identification of pathogenic

E.coli strains needs to detect factors that determine the virulence of these organisms; also it has become possible to detect pathogenic genes in bacterial isolates, allowing the rapid diagnosis of pathogenic *E.coli*. PCR methods using single primer sets have been reported (Oswald et al, 2000).

Aim of work:

Due to the rising incidence of outbreaks associated with *E.coli* infection in growing rabbits. It depends greatly on investigating the causative agents, eliminating them to ensure safety and to protect public health from microbial contamination according to the following points were done:

1. Isolation of *E.coli* from rabbits.
2. Biochemical characterization of *E.coli* isolates from rabbits.
3. Serological identification of isolated strains of *E. coli*.
4. PCR for detection of some virulence genes (*phoA* , *eaeA* , *tsh* and *stx1,2*).

Material and Methods

Samples:

A total number of 125 diseased rabbits collected from different farms in Portsaid Governorate during the period of work from January 2016 till March 2017. 625 collected samples were taken from

liver, heart blood, spleen, kidney and intestine (125 of each).

Bacteriological isolation and identification of *E.coli*:

Isolation and identification of *E.coli* were determined according to Koneman et al., (1997), Cruickshank et al., (1975) and John et al. (1970).

Serotyping of *E.coli* isolates: The diagnostic *E.coli* antisera sets (Denka Seiken Co. LTD, Tokyo, Japan for antisera) were used for identification.

Molecular Identification of *E.coli* Isolates:

A total of 5 presumptive samples of *E.coli* by cultural, morphology and biochemical characteristics, were tested by specific primer employing PCR assay which was more sensitive in the confirmation of the isolates.

Extraction of DNA: It was done according to QIAamp DNA mini kit instructions

Preparation of PCR Master Mix used for cPCR

Oligonucleotide primers used in cPCR

Oligonucleotide Primers used to amplify *E.coli* and its virulence genes are listed in Table (1)

Cycling conditions of cPCR : Temperature and time conditions of the primers during PCR are shown in Table (2).

Table (1): Oligonucleotide primers sequences Source:

| Target gene | Primers sequences | Amplified segment (bp) | Reference |
|-------------|----------------------------|------------------------|-----------------------------------|
| <i>phoA</i> | CGATTCTGGAAATGGCAAAAG | 720 | Hu <i>et al.</i> , 2011 |
| | CGTGATCAGCGGTGACTATGAC | | |
| <i>eaeA</i> | ATG CTT AGT GCT GGT TTA GG | 248 | Bisi-Johnson <i>et al.</i> , 2011 |
| | GCC TTC ATC ATT TCG CTT TC | | |
| <i>Tsh</i> | GGT GGT GCA CTG GAG TGG | 620 | Delicato <i>et al.</i> , 2003 |
| | AGT CCA GCG TGA TAG TGG | | |
| <i>stx1</i> | ACACTGGATGATCTCAGTGG | 614 | Dipineto <i>et al.</i> , 2006 |
| | CTGAATCCCCCTCCATTATG | | |
| <i>stx2</i> | CCATGACAACGACAGCAGTT | 779 | |
| | CCTGTCAACTGAGCAGCACTTTG | | |

Table (2): Cycling conditions of the different primers during cPCR

| Target gene | Primary den. | Sec. den. | Ann. | Ext. | Final ext. |
|---------------|----------------|-----------------|-----------------|-----------------|-----------------|
| <i>phoA</i> | 94°C 5 min. | 94°C 30 sec. | 55°C 45 sec. | 72°C 45 sec. | 72°C 10 min. |
| <i>eaeA</i> | 94°C 5 min. | 94°C 30 sec. | 51°C 30 sec. | 72°C 30 sec. | 72°C 7 min. |
| <i>tsh</i> | 94°C 5 min. | 94°C 30 sec. | 54°C 45 sec. | 72°C 45 sec. | 72°C 10 min. |
| <i>Stx1,2</i> | 94°C 5 min. | 94°C 30 sec. | 58°C 45 sec. | 72°C 45 sec. | 72°C 10 min. |

DNA Molecular weight marker

Agarose gel electrophoresis: (Sambrook *et al.*, 1989)

Results & Discussion

Prevalence of *E.coli* isolated from diseased rabbits.

A total of 125 rabbit, *E.coli* recovered in 105 rabbit with percentage 84% as shown in Table (3).

Seasonal incidence of *Escherichia coli* infection in diseased rabbits: as shown in (Table 4)

Incidence of *E.coli* isolated from different organs of diseased rabbits: as shown in Table (5) Serotypes of *E.coli* recovered from diseased rabbits: as shown in Table (6)

Genotypic characterization of *E.coli* (Conventional Polymerase chain reaction (cPCR) for detection of virulence genes:

A total of five presumptive samples of *E.coli* by cultural, morphology, biochemical and serological characteristics, were tested by specific primer employing PCR assay which was more sensitive in the confirmation of the isolates as shown in Table (7):

Detection of attaching and effacing gene (*eaeA* gene): As shown in **Figure (1)** Lane 1, 3, 4, isolates gave positive electrophoresis of attaching and effacing gene (*eaeA* gene) with a specific band at 248 base pair.

Detection of temperature sensitive hemagglutinin gene (*tsh*):as shown in **Figure (2)** Lane1 isolate gave positive electrophoresis of temperature sensitive hemagglutinin gene (*tsh*) with a specific band at 620 base pair.

Detection of alkaline phosphatase

gene (*PhoA*):

As shown in **Figure (3)** all tested isolates gave positive electrophoresis of alkaline phosphatase gene (*PhoA*) with a specific band at 720 base pair.

Detection of Shiga-Like Toxin gene (*stx1 and stx2*): as shown in **Figure (4)** all tested isolates gave negative electrophoresis of Shiga-Like Toxin gene (*stx1 and stx2*) with a specific band at 779 and 614 base pair respectively.

E. coli is a normal component of rabbit digestive flora and it does not always exert direct pathogenic activity in rabbits. Stress or other pathogen may trigger its overgrowth in the gut environment, which can be resulted in diarrhea or death (*Milon., 1996*). Regarding to prevalence of *E. coli* isolated, the obtained data in Table (3) revealed that percentage of *E. coli* isolation from diseased rabbits was 84%. Similar close rates also were recorded by (*Johnson et al., 2005b; Claudie et al., 2009; Lyhs et al.,2012; Eid and Erfan, 2013 and Peer et al., 2013*) who recovered *E. coli* in 92%, 75%, 94.5%, 80% and 84% of the tested samples respectively while lower rates were recorded by (*Sharada et al., 2010; Hasan et al.,2011; Literak et al., 2013 and Radwan et al., 2014*) who isolated *E. coli* with percentages of 44.61%, 36.20%, 35.74% and 41.5% respectively. Concerning, seasonal prevalence of *Escherichia coli* infection in rabbits, Summer season was found to be the most

important season that influenced diarrhea in rabbits. (92.8%) during summer compared to 86.5% ,80%, 76% during spring, autumn and winter seasons respectively table (4). This result agreed to some extent with **Habeeb et al. (1997)** who showed that, the highest incidence rate of *E.coli* was 18% in summer season, while no isolation of *E.coli* was recorded during winter season. Similarly. **Hussein (2015)** stated that out of 192 rectal fecal swabs collected from diseased rabbits at different seasons, 84 isolates of *Escherichia coli* were recovered and the highest incidence in summer season by percentage 56.9%. In the present study 345 isolates, 105(30.4%) from liver, 100 (29%)from intestine ,60(17.4%) from kidney,50(14.5%)from heart blood and 30(8.7%)from spleen (Table 5). Also, **El-Tabiy (1998)** reported that out of 150 diseased rabbits, 146(26.4%) from intestine, 141(25.5%) from liver, 136 (24.6%) from heart blood and 130(23.5. %) from spleen. Concerning, serological serotyping, 25 *E. coli* isolates recovered from diseased rabbits were belonged to 4 different O serotype groups besides untypable one. The most prevalent serogroup was O153 (28%) followed by O27 (24%), untypable (20%), O125 (16%) and O158 (12%) (Table 6). Similar finding has been reported by **Saad, (1994) and Alshimaa, (2007)** isolated *E. coli* O125 from weaned rabbits. Also, **Aisha and Youseif (1999)** isolated

O128, O125, O158 and untyped strain **Shahin et al., (2011)** isolated *E. coli* serogroup O158 from diarrheic rabbits. **Hussein (2015)** reported that isolated *E. coli* strain from diarrheic rabbit were belonged to O stereotypes in order of frequency O158, O125, O27 and Untypable (20%, 13.33%, 13.33% and 6.67%) isolates respectively.

Out of 25 *E. coli* serotyped isolates, only 5 different serotyped isolates were subjected to PCR for detection of virulence genes (*eaeA*, *tsh*, *phoA* and *stx1*, 2) ,Results of PCR analysis showed that isolated *E. coli* (O153, O185 and O27) strains presented (*eaeA*) gene, with percent 60% of the screened isolates Table(8) and Figure (1). While *tsh* gene present in *E. coli* O153 with percentage 20% Figure (2). but *phoA* gene present in all of the screened isolates (O153,O125, ,O158, O27 and untyped serogroup) with percentage 100% Figure (3). No one contain Shiga-like Toxin1 and 2 producing *E. coli* strain (*stx1* and *stx2*) Figure (4). The results come in agreement with the previously reported by **blanco et al., (1996)** they showed that rabbit O26 strains presented the *eaeA* gene but not any of verotoxin encoding gene. The results are in accordance with the previously reported by **Sabry and Mohamed, (2009)** reported that *E. coli* O26 and O55 isolated from rabbits displayed (*eaeA*) gene and no one produce Shiga-like Toxin. While **Alton et al., (2012)** reported that, fecal

culture examination of 20 rabbits yielded 48 *E. coli* isolates, 83% of which were *eaeA* positive.

Concerning, *tsh* gene, *Ashraf et al.*, (2014) detected *eaeA* and *tsh* genes in three serogroups of *E. coli* (O55, O125 and O146) while *stx2* virulence gene was detected in two serotypes (O55 and O125). *Hussein (2015)* carried out PCR assay for *E. coli* serotypes (O158, O128, O125, O20, O27, O18 and O148) to detect *eaeA* and *tsh* genes. It was found that 100 % (7/7) of tested *E. coli* isolates carry *eaeA* virulence gene, and 87.8 % (6/7) of the tested *E. coli* isolates were positive to *tsh* gene. In another study by *Hagedorn et al. (2011)* who reported that, although *tsh* gene associated with

the bird, it was also found in 46% of *E. coli* isolated from a dog with diarrhea, which leading the authors to propose that, this gene would be a better source tracking marker from faeces of other animals.

Concerning *phoA* gene, *Ke Xin Yu and Kwai Lin Thong (2009)* reported that all *E. coli* strains isolated from environmental water showed positive result for the presence of *phoA* gene, thus confirming their identity as *E. coli*. While *Wei et al. (2013)* given that (86.2%) of isolates recovered from infected ducks were positive for *E. coli PhoA* gene. Most of the isolated *E. coli* serotypes are usually associated with many cases of food borne outbreaks.

Table (3): Prevalence rate of *E. coli* isolated from diseased Rabbits:

| Number of rabbits | Number of +Ve cases | Prevalence of +Ve cases | Number of -Ve cases | Prevalence of -Ve cases |
|-------------------|---------------------|-------------------------|---------------------|-------------------------|
| 125 | 105 | 84% | 20 | 16% |

+Ve= Positive

-Ve= Negative

Table (4): Seasonal incidence of *Escherichia coli* infection in diseased rabbits

| Season | No. of diseased rabbits | Incidence | |
|--------|-------------------------|------------------|------|
| | | No. of +ve cases | % |
| Autumn | 35 | 28 | 80 |
| Winter | 25 | 19 | 76 |
| Spring | 37 | 32 | 86.5 |
| Summer | 28 | 26 | 92.8 |
| Total | 125 | 105 | 84% |

Table (5): Incidence of *E.coli* isolated from different organs of diseased rabbits

| Total No. of Examined samples | liver | | Intestine | | spleen | | kidney | | Heart blood | | Total No. Of isolates | |
|-------------------------------|-------|------|-----------|----|--------|-----|--------|------|-------------|------|-----------------------|------|
| | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % |
| 625 | 105 | 30.4 | 100 | 29 | 30 | 8.7 | 60 | 17.4 | 50 | 14.5 | 345 | 55.2 |

Table (6): Serotypes of *E.coli* recovered from diseased rabbits:

| Serotype | Number | Percentage* |
|-----------|--------|-------------|
| O153 | 7 | 28% |
| O125 | 4 | 16% |
| O27 | 6 | 24% |
| O158 | 3 | 12% |
| Untypable | 5 | 20% |
| Total | 25 | 100% |

Table (7): Genotypic characterization of *E.coli* (Conventional Polymerase chain reaction (cPCR) for detection of virulence genes:

| Virulence genes | <i>E.coli</i> isolates | Percentage |
|-----------------|------------------------|------------|
| <i>PhoA</i> | 5/5 | 100% |
| <i>eaeA</i> | 3/5 | 60% |
| <i>tsh</i> | 1/5 | 20% |
| <i>Stx1,2</i> | 0/5 | 0% |

Table (8): Distribution of virulence genes among (O) serogroups of diarrheagenic *E.coli*.

| serogroup | Genes | | | | |
|-----------|-------------|-------------|------------|-------------|-------------|
| | <i>phoA</i> | <i>eaeA</i> | <i>tsh</i> | <i>Stx1</i> | <i>Stx2</i> |
| O153 | + | + | + | - | - |
| O125 | + | - | - | - | - |
| O158 | + | + | - | - | - |
| O27 | + | + | - | - | - |
| Untyped | + | - | - | - | - |

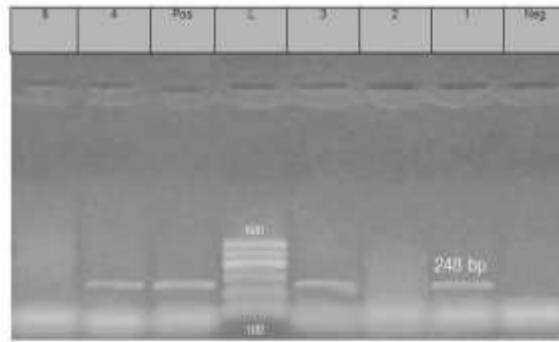
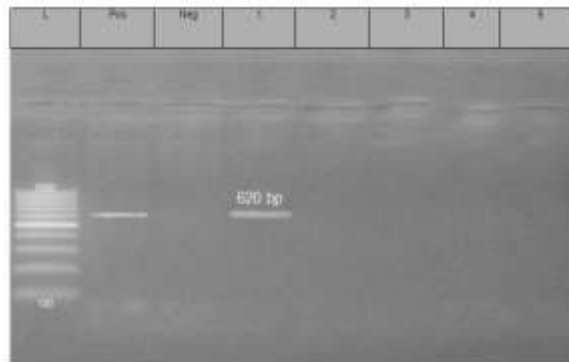


Figure (1): Agarose gel (1%) electrophoresis showing result of PCR for *eaeA* gene .

Lane(1, 3, 4) →positive for *eaeA* gene with 248 bp band.



Figure(2): Agarose gel (1%) electrophoresis showing result of PCR for *tsh* gene.

Lane1 → positive for *tsh* gene with 620 bp band.

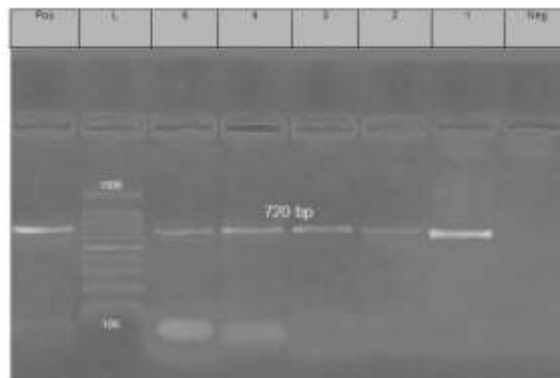


Figure (3): Agarose gel (1%) electrophoresis showing result of PCR for *PhoA* gene.

Lane(1,2,3,4,5) → positive *PhoA* gene with 720 bp band.

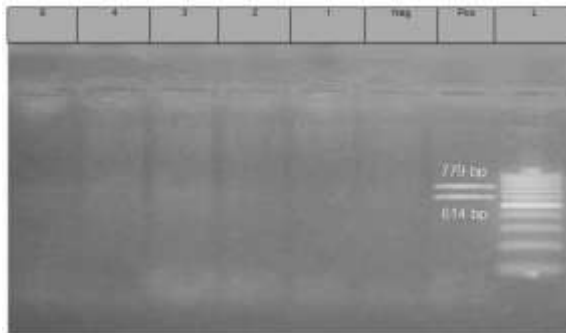


Figure (4): Agarose gel (1%) electrophoresis showing result of PCR *stx1* and *stx2* gene .
Lane (1,2,3,4,5) → negative for *stx1* and *stx2* genes with 779 and 614 bp band respectively.

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الملخص العربي

دراسات بكتريولوجية علي عدوي الإشيريشياكولاي في الأرانب

حمزة محمد عيد **نهله طه عبد الجوادإيمان محمود زغلول**

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**** قسم صحة الأغذية معهد بحوث صحة الحيوان الدقي فرع بورسعيد - -**

*****طبيبة بيطرية**

تم جمع عدد 125 من الأرانب المريضة التي تعاني من الإسهال متوسط عمرها (1-2) شهر من مزارع متعددة للأرانب بمحافظة بورسعيد خلال الفترة من (يناير 2016 - حتى مارس 2017) والتي أظهرت عزل الميكروب القولوني الإشيريشياكولي من 125 أرنب بنسبة 84%. تم تجميع 625 عينه من الكبد والقلب والرئء والكلي والأمعاء كما بينت النتائج ان اعلي نسبة اصابه كانت بالكبد بنسبه (30.4%) يليها الأمعاء بنسبه (29%) يليها الكلي بنسبه (17.4%) يليها القلب بنسبه (14.5%) واقل نسبة اصابه كانت بالطحال بنسبه (8.7%). كما بينت النتائج أن أعلى نسبة إصابة حدثت في فصل الصيف بواقع 28/26 بنسبة (92.8%). تلاها فصل الربيع بمعدل 37/32 بنسبة بلغت (86.5%)، ثم في فصل الخريف 35/28 (80%)، وأخيرا فصل الشتاء 25/19 (76%). وباجراء التصنيف السيرولوجي لمعزولات الميكروب القولوني وجد انها تنتمي الى كل من (O153, O125, O27, O158) وعترات غير مصنفة بنسبة (28% و 16% و 24% و 12% و 20%) على التوالي. كما تم اجراء اختبار انزيم البلمرة المتسلسل التعددي للمعزولات الاتية لتحديد بعض الجينات المسؤولة عن الضراوة (O153, O125, O27, O158) , وقد اوضحت النتائج وجود جين (*phoA*) في جميع العترات كما وجد جين (*eaeA*) ايضا في العترات O153 و O158 و O27 كما وجد جين (*tsh*) ايضا في العتره O153 فقط كما اوضحت النتائج عدم وجود جين (*stx1,2*) في اي من العترات.