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Prevalence of diarrheagenic *Escherichia coli* in suckling rabbits

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Abstract

Diarrheagenic *Escherichia coli* (*E. coli*) in suckling rabbit causes collibacillosis, which is characterized by sever yellow diarrhea, poor growth and high mortalities. This study was undertaken to investigate the prevalence of diarrheagenic *E. coli* in suckling rabbits in Egypt. Additionally, expression of some virulence-associated genes in the isolated *E. coli* serotypes were examined using the polymerase chain reaction. Finally, antibiogram of the identified *E. coli* serotypes was also investigated. *E. coli* could be isolated from 40% of the examined samples collected from diseased suckling rabbits having yellowish diarrhea. Cecum harbored the highest incidence of *E. coli* followed by stomach, liver, heart and spleen. Four serogroups could be identified including *E. coli* O109:H2, O15:H-, O103:H2 and O8:H-. Isolated pathogenic *E. coli* serogroups harbored different virulent factors responsible for diarrhea. Additionally, isolated *E. coli* serogroups showed marked low sensitivity or even resistance to the most common used antibiotics in Egypt.

Key Word: *E. coli*, rabbits, Egypt

Introduction

Collibacillosis caused by Enteropathogenic *E. coli* (EPEC) is an infectious disease of major economic importance in the rabbit industry and may have a high incidence and mortality⁴. Collibacillosis in suckling rabbits is characterized by sever yellow diarrhea, poor growth and high mortalities¹².

Escherichia coli (*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 contact¹¹. 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 pathotype¹⁴.

There is a lack of information about the incidence of *E. coli* in suckling rabbits in Egypt. Thus, the objectives of this study were firstly to investigate the incidence of diarrheagenic *E. coli* in suckling rabbits from rabbitries at Zagazig city, Sharkia governorate, Egypt. Additionally, expression of some virulence-associated genes in the isolated *E. coli* serotypes were examined using the polymerase chain reaction (PCR). Finally, antibiogram of the

identified *E. coli* serotypes was also investigated.

Materials and Methods

Specimens:

A total number of 250 specimens (50 each of cecum, stomach, liver, heart and spleen) were collected from 50 suckling rabbits (moribund or freshly dead) collected from different rabbitries at Zagazig city, Sharkia governorate, Egypt.

Bacteriological examination:

Tissue samples were plated on MacConkey agar plates (Difco, Detroit, MI, USA), and then incubated at 37° C for approximately 24 h in aerobic conditions. The lactose fermenting colonies were re-inoculated to eosin methylene blue agar plates (Difco, Detroit, MI, USA). Colonies producing metallic sheen were transferred to Nutrient agar slants and incubated at 37° C for 24 h and then stored at 4° C for further identification. Identification of isolates was done according to the method described before⁵ based on staining and biochemical tests (Catalase, Oxidase, Indol, Methyl Red, VP test, Citrate utilization, Nitrate reduction, Urease, H₂S production, Gelatin liquefaction and Eijkman test).

Serodiagnosis of E. coli:

The confirmed *E. coli* isolates were serologically identified⁸ by using rapid diagnostic *E. coli* antisera sets (Difco, Detroit, MI, USA) for diagnosis of the Enteropathogenic types.

DNA preparation:

Bacterial DNA was extracted from each of glycerol stock serodiagnosed *E. coli* according to the method described before⁶. DNA concentration in supernatant was evaluated by Nanodrop (ND-1000, Nanodrop Technologies, Wilmington, DE, USA).

Detection of virulence factors by Polymerase Chain Reaction (PCR):

Tested *E. coli* were examined for harboring different virulence-associated genes including episomal increased serum survival protein (*iss*), putative hemolysin (*hlyF*), enteroaggregative toxin (*astA*), and temperature sensitive hemagglutinin (*tsh*) using PCR. The PCR procedure was applied after DNA extraction, according to the protocol described before³. Primers for specific amplification of the tested virulence-associated genes (*iss*, *hlyF*, *astA* and *tsh*), annealing temperatures, fragment size, and accession numbers are described (Table 1) and were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The PCR reaction was started with a single 4 min cycle at 94°C, followed by 40 cycles of 15 sec denaturation at 94°C, 45 sec annealing, and 1 min extension at 72°C and a final extension for 7 min at 72°C. The amplified products were separated by electrophoresis in 1.5% agarose gel and stained with ethidium bromide. The 100 bp DNA ladder was used as a molecular size marker. The specificity of the PCR product was confirmed by direct sequencing of all amplicons using an automated DNA Sequencer (ABI Prism 310 genetic analyzer).

Table (1): Sequence and specificity of PCR primers and their product sizes

No	Virulent genes	Primer sequence (5'-3')	Size (bp)	GeneBank (Acc. No)	Tm°
1	<i>iss</i>	CAGCAACCCGAACCACTTGATG AGCATTGCCAGAGCGGCAGAA	323	X52665	60
2	<i>hlyF</i>	GGCCACAGTCGTTTAGGGTGCTTACC GGCGGTTTAGGCATTCCGATACTCAG	450	FQ482074	67
3	<i>astA</i>	TGCCATCAACACAGTATATCC TCAGGTCGCGAGTGACGGC	116	AF143819	58
4	<i>tsh</i>	GCACGAACTGGGAAGTATGGA GGCATAGAAACCACCACCCC	280	AF218073	68

Antibiogram:

Antibiotic sensitivity test was performed according to the previous procedures²⁾, utilizing nutrient agar plates by placing 20 mm antibiotic discs of 8 commonly used antimicrobial agents and measuring the diameter of zone of inhibition.

Results and Discussion

Escherichia coli is a commensal resident of the intestine as well as a pathogen that can cause both enteric and extraintestinal infections of rabbits¹¹⁾. In this study, we could isolate pathogenic *E. coli* from diseased suckling rabbits with a percentage of 40% (100 samples out of 250 examined samples). In line with this result, *E. coli* was previously isolated from suckling rabbits in different countries such as England, Italy and Portugal¹⁾. Regarding the incidence of *E. coli* in the different tissues, cecum had the highest incidence with a percentage of 66% followed by stomach, liver, heart and spleen with percentages of 44%, 40%, 30% and 20% as clear in figure 1. These results go in line with our previous report⁶⁾, as *E. coli* was also isolated from liver, gizzard, spleen and heart of ducks in Zagazig, Egypt.

Four *E. coli* serogroups could be identified from the diseased rabbits, *E. coli* O109:H2 was the most prevalent serogroup with a percentage of 38%, followed by *E. coli* serotypes O15:H-, O103:H2 and O8:H- with percentages of 27%, 20% and

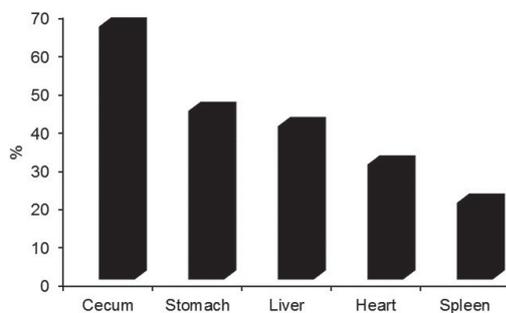


Figure 1: Incidence of *E. coli* in the different tissues of the examined suckling rabbits

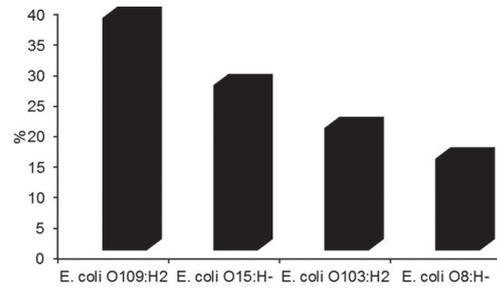


Figure 2: Incidence of different *E. coli* serotypes isolated from the examined suckling rabbits

15% respectively (Fig. 2). Similarly, *E. coli* O109 and O15 frequently isolated from rabbits in Switzerland⁷⁾.

Concerning the expression of the virulence-associated genes, we observed that *iss*, *hly* and *tsh* which are major determinants for diarrhea, hemorrhage and focal intestinal adhesion were expressed in all identified serogroups, while *astA*, which is responsible for toxin production was not expressed in all tested serotypes as declared in figure 3. These results were corresponding to that reported before in *E. coli* isolated from ducks and rabbits^{6,13)}.

Regarding the antibiotic sensitivity of the isolated *E. coli* serogroups, the results recorded in table 2 showed that *E. coli* O109 was highly sensitive to ampicillin, moderately sensitive to amoxicillin, gentamycin and neomycin but weakly sensitive or resistant to ciprofloxacin, danofloxacin, florfenicol and oxytetracycline. *E. coli* O15 was moderately sensitive to ampicillin, amoxicillin and gentamycin but weakly sensitive or resistant to norfloxacin and oxytetracycline respectively. *E. coli* O103 was highly sensitive to amoxicillin, gentamycin and florfenicol but weakly sensitive to ciprofloxacin and oxytetracycline. *E. coli* O8 was moderately sensitive to amoxicillin, ampicillin, neomycin and norfloxacin but sensitive to other tested antibiotics (Table 2). It is noteworthy that these findings were partially correlated to those reported before^{9,10)}.

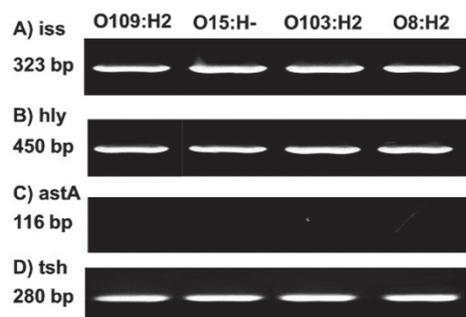


Figure 3: DNA expression of virulence-associated genes in the identified *E. coli* serogroups

Table (2): Antibiogram of various pathogenic *E. coli* serogroups isolated from examined poultry species towards various antibiotics

Antibiotic	O109	O15	O103	O8
Amoxicillin	+++	+++	++++	+++
Ampicillin	++++	+++	+++	+++
Ciprofloxacin	+	++	+	++
Danofloxacin	+	++	++	++
Gentamycin	+++	+++	++++	++
Florfenicol	+	++	++++	++
Neomycin	+++	++	+++	+++
Norfloxacin	++	+	+++	+++
Oxytetracycline	-	-	+	+

(++++) **Highly sensitive**; (++++) **Moderately sensitive**; (++) **Sensitive**; (+) **Weakly sensitive**; (-) **Resistant**

The possible explanation for the low sensitivity of some *E. coli* serogroups against the antibiotics as found in the study could be attributed to the indiscriminate use of these antibiotics in some rabbit farms under the intensive rearing systems. This high resistance might be due to transmissible drug resistance and resistance might also develop due to mutational changes.

Conclusions

E. coli could be isolated from suckling rabbits suffering from diarrhea in some rabbit farms at Zagazig city, Egypt. Four *E. coli* serogroups could

be identified including *E. coli* O109:H2, O15:H-, O103:H2 and O8:H-. Cecum harbored the highest incidence of *E. coli* followed by stomach, liver, heart and spleen. Isolated *E. coli* serogroups harbored different virulent factors responsible for diarrhea. Additionally, isolated *E. coli* serogroups showed marked low sensitivity or even resistance some of the commonly used antibiotics in Egypt.

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