Global Veterinaria 3 (3): 260-267, 2009
ISSN 1992-6197

Determination of Virulence Gene Markers and Antimicrobial Resistance in *Escherichia coli* Isolated from Rabbit in Egypt

Sabry A. Hassan and Mohamed W. Abd Al-Azeem

Microbiology Department, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

Abstract: Fecal samples from 48 diarrheic rabbits, acquired from commercial vendors and households at Qena governorate, Egypt, were cultured for *Escherichia coli*. A total of forty *E. coli* strains were recovered and twenty nine of these, were serotyped, tested with polymerase chain reaction (PCR) for the presence of virulence gene markers of enteropathogenic and toxigenic *E. coli* (*eae*, *bfp*, *stx1*, *stx2*, *LT, ST, cnf1*, *cnf2*) and investigated for antimicrobial resistance. Detection of drug susceptibly was determined using disc diffusion method to ampicillin (AMP), amoxycillin (AMX), chloramphenicol (CHL), enrofloxacin (ENR), flumequine (FLU), streptomycin (STR), sulphamethoxazole (SMX) and tetracycline (TET). Serological identification led to recognition of O26, O55, O103, O128 and O145 serogroups. Amplification of various genes analyzed showed that, 31% (9 of 29) of the tested *E. coli* strains, possess *eae* gene and three displayed toxin genes (one each of *stx1*, *cnf1*, *cnf2*). Gene markers of BFP, LT, ST and Stx2 could not detected. Result of antibiograms have shown that all the isolates were sensitive to different antimicrobials tested. Resistance to one or more antimicrobials was exhibited by 8 strains of the 29 strains examined. Single resistance was found against SMX (three strain), AMP (two strains) and STR (one strain). Multiple resistance was observed to SMX, TET, AMP, CHL, STR (one strain) and SMX, TET, CHL, STR (one strain). Generally, the study demonstrated that rabbit *E. coli* isolates express *eae* gene encoding for intimin among and rare toxin displayed. Rabbit *E. coli* showed general sensitivity to antimicrobials, however some exhibited antimicrobial resistance. Presence of pathotype marker genes, serotypes and resistance in rabbit *E. coli*, could constitute potential source of transmission to human.

Key words: Rabbit diarrhea · Virulence markers · Enteropathogenic *E. coli* · Toxigenic *E. coli* · Serotypes · Antimicrobials PCR

INTRODUCTION

In recent years, there has been increasing commercial production of rabbits as a source of protein. In Egypt, increasing demand for rabbits meat due to its palatable test and popularity. In addition to this commercial value, these animals are used as very important models for medical research and as pets [1]. Rabbits are highly susceptible to diseases and intestinal infections are among the most serious health problems of industrial rabbit production. From an etiological aspect, bacteria, viruses and protozoa are involved. According to Peåters (1984) enteropathogenic *E. coli* bacteria together with clostridia and eimeria, are most commonly implicated in the etiology of diarrhea in rabbits [2]. The enteric diseases that associated with *E. coli* pathogenic strains, are often attributable to intestinal colonization by *E. coli* [3].

Escherichia coli is the most predominant facultative anaerobe of the colonic flora. Diarrheagenic *E. coli* strains include five major groups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC) and enteroinvasive *E. coli* (EIEC) [4,5].

The enteric diseases due to EPEC strains are considered a major cause of weight loss, watery diarrhea and high mortality rates in rabbits. The hallmark of the infection due to EPEC is the attaching and effacing (A/E) histopathologic condition, which is characterized by effacement of microvilli and intimate adherence between the bacterium and the epithelial cell membrane. Intimin, an outer membrane protein, is an adhesin encoded by the *eae* gene (for *E. coli* A/E).

Corresponding Autor: Sabry A. Hassan, Microbiology Department, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

EPEC is currently subdivided into typical and a typical subgroups. While typical EPEC carry the EPEC adherence factor plasmid (pEAE) that encodes the bundle-forming pilus (BFP) and a complex regulatory of various virulence genes (Per) [6], atypical EPEC is devoid of pEAF (or does not express a functional BEP) [6,7]. Enteropathogenic *E. coli* (EPEC) and the majority of clinical isolates of Shiga toxin (Stx)-producing *E. coli* (STEC) harbor the "locus of enterocyte effacement" (LEE), a pathogenicity island that is responsible for the phenotype of attaching-and-effacing (A/E) lesions [6,8].

Rabbit EPEC (REPEC) bacteria constitute a subset of the EPEC pathotype. The virulence properties of rabbit EPEC (REPEC) strains seem to be related to the presence of the *eae* gene, encoding for the protein intimin that is required for the development of the Attaching-Effacing lesions and to the presence of the AF/R1 and AF/ R2 fimbriae [9,10].

In rabbits, most food transformation processes occur in the caecum, which is densely populated with bacteria and constitute a favorable culture system. Digestive disorders are the predominant cause of mortality in commercial rabbits [11]. These diseases have been usually treated by administration of antimicrobial substances. However, such management while decreasing the mortality rates, may allow for selection and spread of plasmid-born resistances among the strains [12]. Of high concern, transmission of drug-resistant strains carrying transmissible genetic elements from animals to humans and stable introduction of novel drug-resistance genes in human pathogens represents a constant, major threat to humans.

E. coli is a frequent component of the gut flora in healthy rabbits, although usually at low rates [13]. Thus, isolation and serogrouping alone are not a sufficient way to the diagnosis of strains pathogenic for rabbits. Furthermore, the experimental reproduction of the disease in healthy rabbits with each field isolate is time-consuming and labor work. Furthermore, in Egypt, there is lack in the information about the virulence characteristics of *E. coli* associated with diarrhea in rabbits.

The present study aim to determine the virulence characteristics of rabbit *E. coli* strains using PCR technique for the presence of virulence gene markers of enteropathogenic *E. coli* (EPEC) and toxigenic *E. coli* pathotypes and to investigate the rabbit *E. coli* for their antimicrobial susceptibility.

MATERIALS AND METHODS

Samples and Bacteriology: Fecal samples from 48 diarrheic rabbits belong to one of commercial vendors and households at Qena governorate in Egypt were incubated overnight at 37°C in buffered peptone water.

Microbiological isolation of *E. coli* was obtained with selective agar medium including MacConkey agar and eosin methylene blue agar (EMB). The *E. coli* suspected colonies were identified by standard biochemical methods [14] and kept at 4±1°C in trypticase soy agar (TSA). Culture media were obtained from BioLab, Hungary. The twenty nine *E. coli* strains a representative of forty strains were used in further analysis.

Serological Identification: A selected *E. coli* strains were subjected to slide agglutination with polyvalent antisera (SIFIN, Berlin, Germany) against somatic antigens of EPEC and toxigenic *E. coli* (O26, O55, O78, O103, O114, O119, O126, O128, O145, O153, O157).

Antimicrobial Susceptibility Test: The selected isolates were tested by disk diffusion method [15]. The bacterial suspension were adjusted in sterile 0.9% saline to 0.5 McFarland standard (10^8 cfu/ml) and spread on Mueller-Hinton agar. The antibiotics tested were chosen based on their use as growth promoters and in human and animal therapeutics, including: ampicillin (AMP-25 µg); amoxicillin (AMX-25 µg); chloramphenicol (CHL-25 µg); enrofloxacin (ENR-5 µg); Flumequine (FLU-30); sulphamethoxazole (SMX-25 µg); streptomycin (STR-10); and tetracycline (TET-30) (MASTDISCS–Mast Diagnostics Ltd., UK). Isolates were evaluated as sensitive, intermediate or resistant according to the inhibition zone diameter [16].

DNA Isolation and Detection of Virulence Genes: Bacterial DNA was isolated using boiling method as previously described [17]. Gene regions coding for the following pathogenic properties were amplified for each bacterial isolate: Shiga-like Toxin 1 and 2 (*stx1*, *stx2*), enteropathogenic attachment and effacement (*eaeA*), bundle-forming pilus (*bfp*), heat-labile toxin (*LT*), heat-stable toxin (*ST*), cytotoxic necrotizing factors 1 and 2 (*cnf1, cnf2*) gene, using specific primers. The amplification was done in a 25 µl containing template DNA, 1.5 mM $MgCl_2$, 10 mM Tris HCL, 50 mM KCL, 0.2 mM of dNTPs, 1 U *Taq* polymerase. The specific primer sequences and the predicted size of the amplified products for the

Table 1: Primer pairs used for detection of the pathotype marker genes used in this study

Primer name	Target gene	Primer sequence ('5 to 3')	Product size (bp)	Reference
eae-1	Intimin (*eaeA*)	ACGTTGCAGCATGGGTAACTC		
eae-2		GATCGGCAACAGTTTCACCTG	815	[22]
BFP-a	bundle-forming pilus (*bfp*)	ATTGGTGCTTGCGCTTGCTGC		
BFP-b		GCCGCTTTATCCAACCTGGTA	326	[23]
STX1-F	Shiga-toxin 1 (*stx1*)	AAATCGCCATTCGTTGACTACTTCT		
STX1-R		TGCCATTCTGGCAACTCGCGATGCA	366	[18]
STX2-F	Shiga-toxin 2 (*stx2*)	CGATCGTCACTCACTGGTTTCATCA		
STX2-R		GGATATTCTCCCCACTCTGACACC	282	[18]
LT-A	Heat-labile toxin (*LT*)	TGTTTCCACTTCTCTTAG		
LT-B		TATTCCCTGTTACGATGT	258	[19]
ST-a	Heat-stable toxin (*ST*)	TCTGTATTATCTTTCCCCTC		
ST-b		ATAACATCCAGCACACAGGC	186	[20]
CNF1-A	Cytotoxic necrotizing factor 1 (*cnf1*)	GAACTTATTAAGGATAGT		
CNF1-B		CATTATTTATAACGCTG	543	[21]
CNF2-A	Cytotoxic necrotizing factor 2 (*cnf2*)	AATCTAATTAAAGAGAAC		
CNF2-B		CATGCTTTGTATATCTA	543	[21]

different pathogenic gene coding regions (Table 1) were employed as previously described [18-23]. PCR was performed with a *PXE*- 0.5 thermal cycler (THERMO, Electron Corporation, Milford, MA, USA) at 94°C for 2 min for 1 cycle followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. The amplified products were visualized by ethidium bromide staining after gel electrophoresis of 10µl of the final reaction mixture in 1.5% agarose. Molecular size markers were included in each gel.

Plasmid Extraction: Plasmid DNA was extracted by alkaline lysis method as previously described [24] and electrophoretic migration was carried out in 0.7% agarose gels and the plasmids were detected by staining with ethidium bromide.

RESULTS AND DISCUSSION

Bacteriology: A worldwide non-coccidial enteric infection of commercially fattened rabbits was increasingly developed [25]. These enteric diseases are often attributable to intestinal colonization by *E. coli* [3]. In this study, all isolated and identified bacteria possessed the morphological and biochemical characteristics of *E. coli*. A total of forty strains were recovered from diarrheic rabbits and twenty-nine of these strains were further characterized. In 1977, Cantey and Blake [26] were the first to isolate from a rabbit a noninvasive strain of *E. coli* (RDEC-1) capable of causing sever diarrhea in this animal species. Previous study in Spain reported that 231 *E. coli* recovered from diarrheic rabbits from different area in Spain and were serotyped, tested for the presence of the *eae* gene and toxin production [3], meanwhile other [27] isolated 105 *E. coli* strains from small intestine, caecum or liver of rabbits coming from a rabbitry located in the province of Teramo (Abruzzi, central Italy) and twenty of these strains were further characterized. In Egypt, bacterial examination resulted in isolation and identification of *E. coli* in 27.64% of diarrheic rabbits [28].

Table 2: Serogroups and virulence marker genes of *E. coli* isolates from rabbit in Egypt (n=29)

Serogroup	N° of isolates (%)	Gene (n° of isolates)
O26	1 (3.4)	*eae*
O55	2 (6.9)	*eae* (1)
O103	6 (20.7)	*eae* (3)
O128	3 (10.3)	*eae* (1)
O145	1 (3.4)	*cnf*1
NT	16 (55.2)	*stx*1 (1); *cnf*2 (1); *eae* (3)

NT: not typable

O-Serogroups: Serogroups found in the 29 *E. coli* strains investigated are shown in Table 2. Using sero-identification, five different serogroups could be demonstrated among the isolates including O26 (one strains), O55 (two strains), O103 (six strains), O128 (three stains) and O145 identified in one isolates, meanwhile the rest of the isolates could not be typable. The O103 and O128 serogroups are included among the

Table 3: Antibiotic resistance patterns showed by *E. coli* strains isolated from rabbits in Egypt (n = 29)

Antimicrobials	No. of isolates (%)
SMX	3 (10.3)
AMP	2 (6.9)
STR	1 (3.4)
SMX, TET, CHL, STR	1 (3.4)
SMX, TET, AMP, CHL, STR	1 (3.4)
Single type resistance	6 (20.7)
Multiple type resistance	2 (6.9)

AMP (ampicillin), CHL (chloramphincol), STR (streptomycin), SMX (sulphamethoxazole), TET (tetracycline)

O serogroups which were more frequently detected in *E. coli* strains isolated from diseased rabbits. Similarly, previous investigation reported that the majority of the *E. coli* strains obtained from diarrheic rabbits belong to only four serobiotypes, whereas the 231 *E. coli* strains isolated from diarrheic rabbits that were investigated, nearly half were of the O103 serogroup [3]. Furthermore, other study in France, showed that More than half of the 575 *E. coli* strains isolated from the cecal contents of weaned rabbits in French commercial farms belonged to serogroup O103 and 70% were of one of five serogroups (O2, O26, O103, O128 and O132) [25]. One *E. coli* strain in this study belongs to EPEC O145 serogroup. Cattle and other ruminants are the main reservoirs of *E. coli* O145 suggested that rabbits may be infected from cattle via contaminated green fodders. In recent report, *E. coli* strain belonged to O145 serogroup were isolated from rabbits, suggested that rabbits may encountered and become infected with EHEC and EPEC strains from rabbit colony contaminated with cattle feces [29].

Experimental infections have shown that *E. coli* strains involved in rabbit enteritis belong to different serotypes [11, 30, 31]. Thus, serotype O109: K2:H2 is mainly associated with yellow diarrhea in suckling rabbits, whereas other serotypes (O2:K1:H6, O15:K2:H2, O20:K2:H7, O26:K2:H11, O103:K2:H2, O109:K2:H7, O128:K2:H2, O132:K2:H2 and O153:K2:H7) are associated with diarrhea in weaned rabbits [32]. However, the epidemiology of EPEC from rabbits remains incomplete, because of the limited number of surveys performed. EPEC strains of serotype O15:K2:H2 are frequently isolated in Belgium, The Netherlands and the United States [11, 30, 33], whereas rhamnose-negative O103:K2:H2 strains are predominant in France and Spain [25, 34, 35]. In Egypt, serological identification of *E. coli* isolates from rabbits led to recognition of O26:K60, O55:K59, O125:K70, O126:K71, O128:K67 [28].

Detection of Drug Resistance: By evaluation of the antimicrobial susceptibility, All isolates were susceptible to FLU, AMX and ENR. Resistance to one or more antimicrobials was exhibited by 8 strains of the 29 strains analyzed. Single resistance was found against SMX (three strain), AMP (two strains) and STR (one strain). Multiple resistance was observed to SMX, TET, AMP, CHL, STR (one strain) and SMX, TET, CHL, STR (one strain) (Table 3).

Rabbit *E. coli* strains had a good sensitivity against quinolones (FLU, ENR) [36]. In our investigation, all isolates exhibited 100 % sensitivity to flumequine (FLU), enrofloxacin (ENR) and amoxicillin (AMX). Similarly, others determined a high sensitivity to quinolones (enrofloxacin and norofloxacin) [37, 38]. However, in the same study, determined the highest resistance percentage against amoxicillin [38]. The data evidenced resistance of rabbit *E. coli* strains against sulphmethoxazole, ampicillin, streptomycin, chloramphincol and tetracycline. Our data similar with regard to these antibiotics to the previously reported [37]. In contrary, [38], reported a preserved sensitivity against chloramphincol.

Virulence Genes: *E. coli* is a normal component of rabbit digestive flora and it does not always exert direct pathogenic activity in rabbits. Stress or other pathogens may trigger its overgrowth in the gut environment, which can result in diarrhea or death [39]. In addition, pathogenicity of some strains may be enhanced by the presence of virulence genes. In this study, we have used the PCR technique as an approach to detect genes encoding intimin, bundle-forming pilus, enterotoxins, Shiga-toxins and cytotoxic necrotizing factors.

Not all *E. coli* strains that cause diarrhea in farm animals synthesize LT or STa enterotoxins. These animal enteropathogenic *E. coli* strains attach to and efface the microvilli of gut epithelium [3]. In rabbits, only this type of *E. coli* enteritis is known to be important and it is caused by EPEC strains, also called AEEC [40]. Our results revealed negative detection of genes encoding LT and ST enterotoxins. Similarly, past investigation showed that non of the *E. coli* strains produced thermostable or thermolabile enterotoxins [34]. For instance, the *eae* gene, that encodes for intimin, is required to allow the development of *Attaching-Effacing* lesions (A/E) [41, 42].

The *eae* gene responsible for the A/E lesions are located on an 35-kb pathogenicity island, known as the locus of enterocyte effacement (LEE). In the present study, amplification of the *eae* gene showed that 31% (9 of 29) of the *E. coli* strains isolated from diarrheic

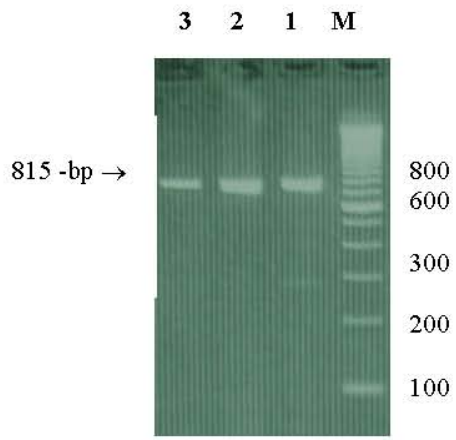

Fig. 1: Agarose gel electrophoresis of PCR amplified product of *eae* gene (815 bp), Lanes: M, DNA Ladder (100bp); 1-3, *eaeA*-positive strains.

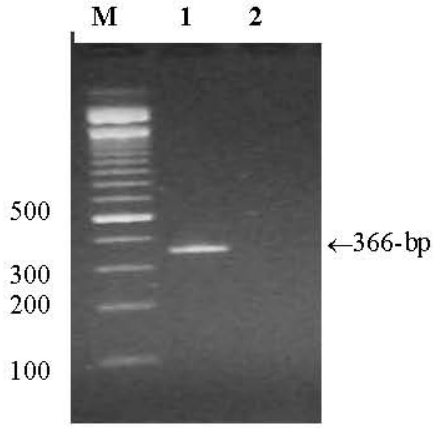

Fig. 2: Agarose gel electrophoresis of PCR amplified product of *stx1* gene (366 bp), Lanes: M, DNA Ladder (100bp); 1, *stx1*-positive strains; 2, *stx1*-negative strain.

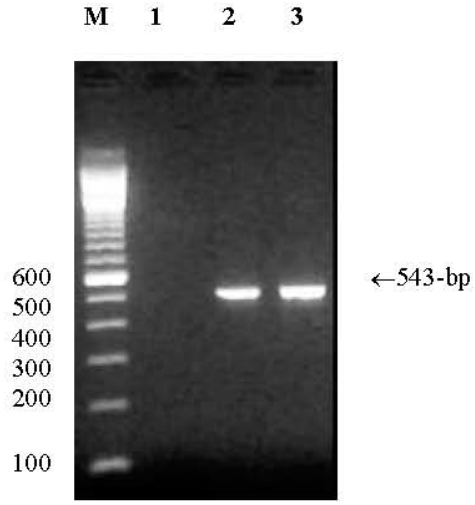

Fig. 3: Agarose gel electrophoresis of PCR amplified product of *cnf1*and *cnf2* (543 bp), Lanes: M, DNA Ladder (100bp); 1, negative strain; 2, 3 cnf-positive strains.

rabbits, possess this virulence determinant (Table 2), (Fig 1). Our results are in agreement with the previous studies on rabbits [3, 37, 41, 43] and showed that *eae* gene, specific for EPEC, was frequently detected among rabbit *E. coli* strains. Sequences homologous to the *eae* gene have been detected in atypical enteropathogenic *E. coli* (AEEC) strains isolated from diarrheic rabbits [41, 43].

Enteropathogenic *E. coli* (EPEC) is currently subdivided into typical and atypical subgroups. Typical EPEC harbor an additional 60 MDa plasmid, the EPEC adherence factor (EAF) plasmid [44], that is not present in atypical EPEC (ATEC) strains [7, 45]. The EAF plasmid harbors the bundle-forming pilus (*bfp*) operon, encoding the type IV pili responsible for localized pattern of adherence (LA). ATEC strains harbor homologues of the LEE pathogenicity island but, due to the lack of the EAF plasmid [45, 46], they most often expresses the LA-like pattern (adhere in a diffuse pattern). Our results confirm those previously reported [47] and show that, to date, no rabbit EPEC (REPEC) strains harboring the *bfp* genes responsible for the localized adherence of human EPEC has been identified. However, one study reported one rabbit *E. coli* strain, belonging to serogroup O103, harbored a self-transferable 117-kb plasmid encoding both antibiotic resistance and virulence determinants [48].

Atypical *E. coli* (AEEC) strains that cause diarrhea in rabbits apparently display a pathogenic mechanism similar to that displayed by human EPEC and enterohemorrhagic *E. coli* strains, some authors have suggested that AEEC strains can synthesize verotoxins (also called Shiga-like toxins) or other types of related cytotoxins [49].

From all of the *E. coli* strains screened, only three were toxigenic: one Shiga-toxin-producing *E. coli* strain (STX1) and two necrotoxigenic *E. coli* strains (CNF1 and CNF2) (Table 2) (Fig 2, 3).

Our results indicate that *E. coli* strains from rabbits rarely produce Shiga-toxins (STX1 and STX2) nor the recently discovered cytotoxic necrotizing factors (CNF1 and CNF2) elaborated by clinical *E. coli* isolates of human and bovine origin [21, 50, 51, 52]. In previous studies, toxigenic *E. coli* strains were rarely identified among rabbit *E. coli* strains. Of a collection of 40 rabbit *E. coli* isolates examined, one O26:B13 strain which reacted

with a VT1 probe [43]. Also, O128:B30 strain has been identified as a producer of cytolethal distending toxin [32].

Of relevance to the *E. coli* strains of serotypes O26 and O55 isolated from rabbits with diarrhea. These two serotypes are considered enterohemorrhagic *E. coli* (EHEC) rather than EPEC serotypes, because many *E. coli* O26 and O55 strains that cause diarrhea in humans and animals produce verotoxins [4, 53]. These two *E. coli* serogroups isolated from rabbits in this study, were negative for VT1 and VT2 by PCR (Table 2), however, displayed *eae* gene similar to human EPEC strains. Our results are in accordance with the previously reported [3] and showed that rabbit O26 strains presented the *eae* gene but not any of verotoxin encoding genes. In Egypt, one study on rabbits diarrhea, detected O26:K60, O55:K59 and O126:K71 serogroups [28]. On the other hand, previous report identified verotoxin and intimin positive *E. coli* in rabbits and indicated that rabbits are a new reservoir host of EHEC that may pose a zoonotic risk for humans [29].

In summary, of the virulence gene markers screened, the *eae* gene was most commonly detected and rarely the toxins encoding genes. Also, when evaluating antimicrobial susceptibility, quinolons drug were shown to efficient against rabbit *E. coli*. In addition, the demonstration of some *E. coli* serotypes, pathotypes and resistant strains among rabbit *E. coli*, may represent a potential source of human infection.

ACKNOWLEDGEMENT

We are deeply grateful to Prof. Ahmed I. Ahmed, head of poultry diseases department, Faculty of Veterinary Medicine, South valley University, for advise, samples collection and manuscript revision. Also, we would like to express our deepest gratitude to Prof. Youssuf A. Gherbawy, department of Botany, Faculty of Science, South Valley University for lab facilities and technical help.

REFERENCES

1. Yousif, O.M.A. and J.A.M. Abdul-Aziz, 1995. Commercial rabbit production. Published by Al-Dar Al-Arabia, Cairo, Egypt.
2. Peeters, J.E., P. Phol and G. Charlier, 1984. Infectious agents associated with diarrhea in commercial rabbits; field study. Annales de Recherches Vétérinaires (Annales in Veterinary Research), 15: 24-29.
3. Blanco, J.E., M. Blanco, J. Blanco, A. Mora, L. Balaguer, M. Mourino, A. Juarez and W.H. Jansen, 1996. O serogroups, biotypes and *eae* genes in *Escherichia coli* strains isolated from diarrheic and healthy rabbits. Journal of Clinical Microbiology, 34: 3101-3107.
4. Levine, M.M., 1987. *Escherichia coli* that cause diarrhea: enterotoxigen, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. Journal of Infectious Diseases, 155: 377-389.
5. Nataro, J.P. and J.B. Kaper, 1998. Diarrheagenic *Escherichia coli*. Journal of Clinical Microbiology, 11: 142-201.
6. Kaper, J.B., J.P. Nataro and H.L.T. Mobley, 2004. Pathogenic *Escherichia coli*. Natural Review in Microbiology, 2: 123-140.
7. Trabulsi, L.R., R. Keller and T.A.T. Gomes, 2002. Typical and atypical enteropathogenic *Escherichia coli*. Emerging Infectious Diseases, 8: 508-513.
8. Dobrindt, U., B. Hochhut, U. Hentschel and J. Hacker, 2004. Genomic islands in pathogenic and environmental microorganisms. Natural Review in Microbiology, 2: 414-424.
9. Pillien, F., M. Chalareng, C. Boury, J. Tasca, J. De Rycke and A. Milon, 1996. Role of Adhesive Factor/Rabbit 2 in experimental enteropathogenic *Escherichia coli* 0103 diarrhea of weaned rabbit. Veterinary Microbiology, 50: 105-115.
10. Penteado, A.S., L.A. Ugrinovich, J. Blanco, M. Blanco, J.E. Blanco, A. Mora, J.R.C. Andrade, S.S. Correa, D.E. Pestana, A.F. and Castro, 2002. Serobiotypes and virulence genes of *Escherichia coli* strains isolated from diarrheic and healthy rabbits in Brazilian Veterinary Microbiology, 89: 41-51.
11. Peeters, J.E., R. Geeroms and F. Ørskov, 1988. Biotype, serotype and pathogenicity of attaching and effacing enteropathogenic *Escherichia coli* strains isolated from diarrheic commercial rabbits. Infection and Immunity, 56: 1442-1448.
12. Vila, J., M. Vargas, C. Casals, H. Urassa, H. Mshinda, D. Schellemberg and J. Gascon, 1999. Antimicrobial resistance of diarrheagenic *Escherichia coli* isolated from children under the age of 5 years from Ifakara, Tanzania. Antimicrobial Agents and Chemotherapy, 43: 3022-3024.
13. Gouet, P. and G. Fonty, 1979. Changes in the digestive microflora of holoxenic rabbits from birth until adulthood. Ann. Biol. Anim. Biochim. Biophys 19: 553-566.

14. Finegold, S.M. and J.W. Martin, 1982. Diagnostic microbiology, 6th. Ed. C.V. Mosby Co. London, U.K.
15. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45: 493-496.
16. [NCCLS] National Committee for Clinical Laboratory Standards, 2004. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, Approved standard (M31-A2), Wayne, PA.
17. Reischl, U., M.T. Youssef, J. Kilwinski, N. Lehn, W.L. Zhang, H. Karch and N.A. Strobine, 2002. Real time fluorescence PCR assays for the detection and characterization of Shiga toxin, Intimin and enterohemolysin genes from Shiga toxin-producing *E. coli*. Journal of Clinical Microbiology, 40: 2555-2565.
18. Brian, M.J., M. Frosolono, B.E. Murray, A. Miranda, E.L. Lopez, H.F. Gomez and T.G. Cleary, 1992. Polymerase chain reaction for diagnosis of enterohemorrhagic *Escherichia coli* infection and hemolytic-uremic syndrome. Journal of Clinical Microbiology, 30: 1801-1806.
19. Lang Lee, A., Yu-Li. Tsai, C.L. Mayer, K.C. Patton and C.J. Palmer, 1994. Multiplex PCR for detection of the heat-labile toxin gene and Shiga-like toxin I and II genes in *E. coli* isolated from natural waters. Applied and Environmental Microbiology, 60: 3145-3149.
20. Schultz, C., G.J. Pool, R. Van Ketel, B. de Wever, P. Speelman and J. Dankert, 1994. Detection of ETEC in stool samples by using nonradioactively labeled oligonucleotide DNA probes and PCR. Journal of Clinical Microbiology, 32: 2393-2397.
21. Blanco, M., J.E. Blanco, J. Blanco, M.P. Alonso, C. Balsalobre, M. Mourino, C. Madrid and A. Juárez, 1996. Polymerase chain reaction for detection of *Escherichia coli* strains producing cytotoxic necrotizing factor type 1 and 2 (CNF1 and CNF2). Journal of Microbiological Methods, 26: 95-101.
22. Gannon, V.P.J., M. Rashed, R.K. King and E.J. Golsteyn Thomas, 1993. Detection and characterization of the *eae* gene of Shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. Journal Clinical Microbiology, 31: 1268-1274.
23. Yatsuyanagi, J., S. Saito, H. Sato, Y. Miyagima, K.I. Amano and K. Enomoto, 2002. Characterization of enteropathogenic and enteroaggregative *Escherichia coli* isolated from diarrhoeal outbreaks. Journal of Clinical Microbiology, 40: 294-297.
24. Kado, C.I. and S.T. Liu, 1981. Rapid procedure for detection and isolation of large and small plasmids. Journal of Bacteriology, 145: 1365-1373.
25. Camguilhem, R. and A. Milon, 1989. Biotypes and O serogroups of *Escherichia coli* involved in intestinal infections of weaned rabbits: clues to diagnosis of pathogenic strains. Journal of Clinical Microbiology, 27: 743-747.
26. Cantey, J.R. and R.K. Blake, 1977. Diarrhea due to *Escherichia coli* in the rabbit, a novel mechanism. Journal of Infectious Diseases, 135: 454-462.
27. Tonelli, A., P. Badagliacca, G. Bruant, A. Letizia, A. Di Provvido, J. Harel and M. Scacchia, 2008. Genetic characterization of rabbit *Escherichia coli* strains with the use of microarray technology. 9th World Rabbit Congress, Verona-Italy, pp: 1097-1101.
28. Abdel-Nasser, A.H.T., L. Ali, Z.A. Hassanein and A.E. Saad, 1998. Current status on bacterial and parasitic agents involved in rabbit enteritis in Northern Egypt. (http://www.cbabstractsplus.org/abstract).
29. García, A. and J.G. Fox, 2003. The Rabbit as a new reservoir host of enterohemorrhagic *Escherichia coli*. Emerging Infectious Diseases, 9: 1592-1597.
30. Peeters, J.E., G.J. Charlier and P.H. Halen, 1984. Pathogenicity of attaching effacing enteropathogenic *Escherichia coli* isolated from diarrheic suckling and weanling rabbits for newborn rabbits. Infection and Immunity, 46: 690-696.
31. Peeters, J.E., P. Pohl, L. Okerman and L.A. Devriese, 1984. Pathogenic properties of *Escherichia coli* strains isolated from diarrheic commercial rabbits. Journal of Clinical Microbiology, 20: 34-39.
32. Peeters, J.E., 1994. *Escherichia coli* infections in rabbits, cats, dogs, goats and horses, pp: 261-283. *In* C. L. Gyles (ed.), *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, Oxon, U.K.
33. Percy, D.H., C.A. Muckler, J. Hampson and M.L. Brash, 1993. The enteritis complex in domestic rabbits: a field study. Canadian Veterinary Journal, 34: 95-102.
34. Blanco, J.E., M. Blanco, J. Blanco, L. Rioja and J. Ducha, 1994. Serotypes, toxins and antibiotic resistance of *Escherichia coli* strains isolated from diarrheic and healthy rabbits in Spain. Veterinary Microbiology, 38: 193-201.

35. Leroy-Setrin, S., M.C. Lesage, E. Chaslus-Dancla and J.P. Lafont, 1995. Clonal diffusion of EPEC-like *Escherichia coli* from rabbits as detected by ribotyping and random amplified polymorphic DNA assays. Epidemiology and Infection, 114: 113-121.
36. Pisoni, A.M., A. Piccirillo, D. Gallazzi, F. Agnoletti and G. Grilli, 2004. Byotype and susceptibility to antimicrobial agents of rabbit *Escherichia coli*. 8th World rabbit congress, Puebla, Mexico, pp: 608-613.
37. Camarda, A., D. Pennelli, P. Battista, V. Martella, L. Greco, I. Allogio and E. Mazzolini, 2004. Virulence genes and antimicrobial resistance patterns of enteropathogenic *Escherichia coli* from rabbits in southern Italy. 8th World rabbit congress, Puebla, Mexico, pp: 470-476.
38. Urumova, V. and V. Petrov, 2008. Analysis of antimicrobial drug resistance in enteropathogenic *Escherichia coli* (EPEC) isolated in rabbits with diarrheic syndrome. Trakia Journal of Sciences, 6: 36-40.
39. Milon, A., 1996. Weaned rabbit colibacillosis: a model for study of enteropathogenic *Escherichia coli*. 6th World Rabbit Congress, Toulouse, pp: 13-22.
40. Moon, H.W., S.C. Whipp, R.A. Argenzio, M.M. Levine and R.A. Giannella, 1983. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. Infection and Immunity, 41: 1340-1351.
41. Leroy, S.M, M.C. Lesage, E. Chaslus-Dancla and J.P. Lafont, 1994. Presence of *eae*A sequences in pathogenic and non-pathogenic *Escherichia coli* strains isolated from weaned rabbits. Journal of Medical Microbiology, 40: 90-94.
42. Agin, T.S., J.R. Cantey, E.C. Boedecker and M.K. Wolf, 1996. Characterization of the *eae*A gene from rabbit enteropathogenic *Escherichia coli* RDEC-1 and comparison to other *eae*A genes from bacteria that cause attaching effacing lesions. FEMS Microbiology Letters, 144: 249-258.
43. Pohl, P.H., J.E. Peeters, E.R. Jacquemin, P.F. Lintermans and J.G. Mainil, 1993. Identification of *eae* sequences in enteropathogenic *Escherichia coli* strains from rabbits. Infection and Immunity, 61: 2203-2206.
44. Levine, M.M., J.P. Nataro, H. Karch, M.M. Baldini, J.B. Kaper and R.E. Black, M.L. Clements and A.D. O'Brien, 1985. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. Journal of Infectious Diseases, 152: 550-559.
45. Beinke, C., S. Laarmann, C. Wachter, H. Karch, L. Greune, M.A. Schmidt, 1998. Diffusely adhering *Escherichia coli* strains induce attaching and effacing phenotypes and secrete homologs of Esp proteins. Infection and Immunity, 66: 528-539.
46. Gärtner, J. and M.A. Schmidt, 2004. Comparative analysis of locus of enterocyte effacement pathogenicity islands of atypical enteropathogenic *Escherichia coli*. Infection and Immunity, 72: 6722-6728.
47. Robins-Browne, R.M., A.M. Tokhi, L.M. Adams, V. Bennett-Wood, A.V. Moisidis, E.O. Krejany, L.E. O'Gorman, 1994. Adherence characteristics of attaching and effacing strains of *Escherichia coli* from rabbits. Infection and Immunity, 62: 1584-1592.
48. Reynaud, A., M. Federighi, D. Licois, J.F. Guillot and B. Joly, 1991. R plasmid in *Escherichia coli* O103 coding for colonization of the rabbit intestinal tract. Infection and Immunity, 59: 1888-1892.
49. Okerman, L., 1987. Enteric infections caused by non-enterotoxigenic *Escherichia coli* in animals: occurrence and pathogenicity mechanisms. A review. Veterinary Microbiology, 14: 33-46.
50. Karmali, M.A., 1989. Infection by verocytotoxin-producing *Escherichia coli*. Clinical Microbiological Review, 2: 15-38.
51. De Rycke, J., E.A. Gonza´lez, J. Blanco, E. Oswald, M. Blanco and R. Boivin, 1990. Evidence for two types of cytotoxic necrotizing factor in human and animal clinical isolates of *Escherichia coli*. Journal of Clinical Microbiology, 28: 694-699.
52. Blanco, M., J. Blanco, J.E. Blanco and D.J. Ramos, 1993. Enterotoxigenic, verotoxigenic and necrotoxigenic *Escherichia coli* isolated from cattle in Spain. American Journal of Veterinary Research, 54: 1446-1451.
53. Hassan, S.A., A. Deutz and M. Awad-Masalmeh, 2006. Virulence factors, O-serogroups and antibiotic resistance of *Escherichia coli* isolates from cases of bovine acute mastitis in Austria. Veterinary Medicine Austria/Wiener Tieraerztliche Monatsschrift, 91: 47-55.