Molecular differentiation of field E. coli strains and local vaccinal strain (Entero-3)

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Abstract
Enterotoxigenic E. coli (ETEC) is the cause of calf morbidity and mortality in addition to zoonotic importance. Vaccination against these causes is strictly required. STEC isolates were isolated from faecal calf samples, biotyped according to biochemical tests and serotyped. STEC genes amplified and detected through PCR. Local vaccinal strain (K99) was included in PCR studies. Serotypes O157:H7 and O127:H6 were commonly isolated from field isolates. Intimin gene was amplified and detected in all isolates, field isolates and vaccinal strain showed the same size band.

Introduction
Diarrhoea is considered to represent a problem that may lead to great economical losses as it consider the most important cause of calf morbidity and mortality specially among newly born calves in Egypt and all over the world (Byomi et al., 1996). Escherichia coli are normal inhabitant of the intestinal tract of the animals. However, particular E. coli strains such as enterotoxigenic E. coli are the causative agents of septicemic colibacillosis and diarrhea (Gyles, 1994). The role of E. coli as pathogen is well known and many E. coli isolates have been associated with a wide variety of diseases. Only limited number of E. coli strains possessing virulence factors that usually complex and mainly associated with the capacity of E. coli to attach and colonize at the site of the infection with elaboration of enterotoxin or by subsequent damage to the host to interfere with its defense (Burrows et al., 1985). K99 antigen is a virulence factor of E. coli strains that are enteropathogenic for calves (Moon et al., 1980). K99 and enterotoxigenicity are controlled by different plasmids (Guinee et al., 1976). Biochemical and serological tests play main role in identification of the field isolates and in the comparison with the local vaccinal strain.

Shiga-toxin-producing Escherichia coli (STEC), also called vero-toxin-producing E. coli (VTEC), is the most important recently emerged group of food-borne pathogens. These bacteria can cause severe disease in humans, such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS).
Cattle, especially young animals, have been implicated as a principal reservoir of STEC, undercooked ground beef and raw milk being the major vehicles of food-borne outbreaks. Human and bovine STEC elaborate two potent phage-encoded cytotoxins, called Shiga-toxins (Stx1 and Stx2) or verotoxins (VT1 and VT2) (Paton and Paton, 1998). In addition to toxin production, another virulence-associated factor expressed by STEC is a protein called Intimin, which is responsible for intimate attachment of STEC to the intestinal epithelial cells, causing attaching intimately and effacing (A/E) lesions in the intestinal mucosa. Intimin is a virulence factor (bacterial outer membrane adhesion molecule) of EPEC (e.g. E. coli O127:H6) and EHEC (e.g. E. coli O157:H7) E. coli strains (Zhao et al., 1995). It is an attaching and effacing (A/E) protein which with other virulence factors is responsible for enteropathogenic and enterohaemorrhagic diarrhoea. Intimin is expressed on the bacterial cell surface where it can bind to its receptor Tir (Translocated intimin receptor). Tir, along with over 25 other bacterial proteins, is secreted from attaching and effacing E. coli directly into the cytoplasm of intestinal epithelial cells by a Type III secretion system. Once within the cytoplasm of the host cell, Tir is inserted into the plasma membrane, allowing surface exposure and intimin binding (Blanco et al., 2004; Kim et al., 2005 and Fagan et al., 1999). After identification by motility, biochemical tests and agglutination tests, PCR assay carried out on the E. coli isolates using eae primers for amplification of Intimin gene with the positive results for all isolates with specific amplification size of 890 bp. However, not all pathogenic STEC isolates have been shown to produce Intimin. The aim of this study was to identify the Enteropathogenic Escherichia coli (EPEC) on the basis of the presence or absence of Intimin gene where E. coli is a dominant bacterial agent of diarrhea diseases in calves; adherence to mucosa is the initial, essential step in the development of pathogenicity for diarrhea associated E. coli (Klemm, 1985).

PCR is probably a useful diagnostic tool for the identification of enterotoxigenic, attaching and toxin producing E. coli strains by amplifying genes encoding K99 enterotoxin and Intimin, the assay was specific and more sensitive. It will be useful for identification of E. coli strains associated with diarrhea in calves (Sophia et al., 1998). A chromosomal gene eaeA encodes the protein Intimin, which involved in attaching to intestinal epithelium (Jerse et al., 1990).

The commercial local vaccine contains (K99) producing E. coli strain which has the proper ability to adhere to intestinal epithelium and its filamentous structure. Calves can be passively protected against enteric colibacillosis if their dams were vaccinated before calving (Nagy, 1980), the local vaccine gave satisfactory results.
The objective of this study was to characterize the field isolates with that of vaccinal strains using PCR assay.

Material and methods

Field samples
A total of 100 faecal swabs were collected from diarrheic calves of 1 day to 12 weeks of age. These calves belong to private and governmental farms. Faecal swab were transported to the laboratory in a cold chamber container to be cultured at same day of sampling.

Standard strain of E. coli
Standard serotype O101:K99 of E. coli obtained from Aerobic Bacterial Vaccine Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. Both field and standard strain were subjected to biochemical, serological identification and PCR.

Bacterial growth
Faecal samples were cultured in nutrient broth then loopful were taken and cultivated on 5% sheep blood agar, MacConkey's agar and EMB (Eeosin methylene blue) agar media as a specific media and incubated at 37°C for 24 hours. The suspected colonies were examined morphologically, microscopically and biochemically according to (Quinn et al, 1994 and Koneman et al, 1997).

Biochemical tests:
Vogas proskour, citrate, hydrogen sulphide, methyl red and indol tests were carried out according to (Ramisse et al, 1984).

Serological identification of isolates:
The suspected colonies were taken from the solid media and subjected to:
1. Slide agglutination test
2. Tube agglutination test
They carried out according to Sojka (1965).

A total of 12 biochemically and serologically identified as E. coli isolates. Diagnostic antisera:
They were obtained from the Veterinary Academy of Moscow by El-Shinawy, Dokki, for identification of isolates of E. coli.

PCR technique (Fagan et al, 1999):
PCR assays were carried out in a 50 ul volume containing 4 ul of nucleic acid template prepared from cultures of field isolates and standard strains and using the following Intimin primers:
Forward:
GTGGGGAAATACTGGGGAGACT
Reverse:
CCCCATTCTTTTTCACCGTCG

PCR mixture consists of 10mMtris-Hcl (PH 8.4), 10 mM Kcl, 3mM Mgcl₂, 20 Pmol of each primer, 0.2 mM dNTPs and one unit of Taq polymerase (Promega, USA) this mix well added to nucleic acid template. The mixture was cycled 35 times through a regimen of 20 second at 95°C, 20 second at 58°C, and 90 second at 72°C under this was accompanied with an initial denaturation for 3 min at 95°C and final extension cycle for 5 min at 72°C. The amplified products were separated by electrophoresis in a 2.5% Metaphor agarose gel (FMC) in the presence of 0.53 Tris-borate-EDTA and were visualized by staining with ethidium bromide under UV light. The results
were recorded by photographic methods.

Results and discussion
The biochemical character of E. coli isolates recovered from the diarrhoeic cases were specified as typical E. coli, they were positive for indol and methyl red while they were negative for Vogas Proskour, citrate and hydrogen sulphide. Meanwhile, they were characterized by classical procedures (acid and gas production from lactose. These results are in agreement with that of (Ramisse et al, 1984).

Dealing with the serological typing of E. coli, it is evident from the results achieved in table (1) that E. coli isolates from diarrhoeic cases were belonged to serotype O157:H7 and O127:H6. These findings are nearly similar to those proved by Lariviere et al (1978), Kaura (1990) and Blanco et al (2003).

Table (1): Detection of adherent E. coli factors

<table>
<thead>
<tr>
<th>Serotypes (O:K)</th>
<th>Source of isolates</th>
<th>K99</th>
<th>H7</th>
</tr>
</thead>
<tbody>
<tr>
<td>O158:K.</td>
<td>Diarrhoeic</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>O127:K63</td>
<td>Diarrhoeic</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>O1:K11</td>
<td>Diarrhoeic</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O157:K.</td>
<td>Diarrhoeic</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O20:K17</td>
<td>Diarrhoeic</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Untypable strain</td>
<td>Diarrhoeic</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All E. coli isolates were examined serologically by K99 antisera. This antigen is known to be important virulence factors expressed by E. coli enabling them to adhere to host tissue (Moon and Bunn, 1993). Both field isolates and standard E. coli strain that used in the production of the commercial vaccine were K99 positive, E. coli K99 strains were isolated in different proportion in different studies as (Tsunemit et al, 1986) who detected 24 K99 E. coli strains from the total of 51 calves.

DNA extraction and PCR assay for eaeA gene amplification were performed as previously described by Kim et al (2005). Oligonucleotide sequences of primers were designed according to (Fagan et al, 1999) and the predicted sizes of PCR amplified products was at 890 bp results of PCR assay for the E. coli isolates showed that all isolates included in this study were have the eaeA gene or in the other word all this strains were enteropathogenic E. coli (EPEC) (photo 1).

Photo (1): Results of PCR on field isolate and reference strains

References


مقارنة جزيئية بين عترات الميكروب القولوني المعدى المستخدم في اللقاح المحلي (أنتيرو-3) والعترات المعزولة محلياً

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المعمل المركزى للقابة على المستحضرات الحيوية البيطرية - عباسية - القاهرة

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تم استخدام اختبار تفاعل البلمرة المتسلسل للتعريف على ميكروب القولون المعدى المفرزة للسموم المعوية سواء كانت معوزلات خلقيّة أو مرجعية مستخدمة في تحضير التحصين المحلي وذلك نظراً لحساسية ودقة *Intimin* للكشف عن أنتيجين الأنتيمين *eaeA* المشترك للكلينيكي والمرجعي. تم استخدام PCR هذا الاختبار وتم استخدام البادئ الجينى *eaeA* الموجود في هذه النوعية من البكتيريا. أيضًا تم عمل الاختبارات الكيميائية والسيرولوجية لعدد أثني عشر عترة من مجموعة مختومة حالة مشاهدة. تم تعريف المعزولات والعترات المستخدمة في هذه الدراسة باستخدام اختبار PCR. كما نوصى اختبار PCR E. coli O127:H6، E. Coli O157:H7، كما نسبة توافق بين العترات المعزولة والعترة المستخدمة في إنتاج اللقاح.