Microbiological Study of Escherichia Coli in Sheep

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ABSTRACT

This study was carried-outona total of 53 rectal swabs samples that were collected from diarrheic sheep of different localities in Behera Governorate. The samples classified into three groups. The first group consists of 40 sheep aged from 1 to 6 months, while the 2nd group consists of 7 sheep aged from 7 to 12 month where the 3rd group consists of 6 sheep were over one year old. The samples were collected, labeled and transported in ice box to the laboratory of Department of Microbiology, Faculty of Veterinary Medicine; Alexandria University. The bacterial isolates were subjected for characterization by cultural, morphological, cultural characteristics, detection of motility, biochemical and by its activities. Out of the 53 samples examined 16 were positive for E. coli isolation (30.2%) all of them (100%) have hemolytic activity and serotyping.

INTRODUCTION

Ruminants, especially cattle and sheep, have been implicated as a principal reservoir of one of the entero-virulent Escherichia coli pathotypes and large and small ruminants could be a potential source of infection in humans with E. coli (Osman et al., 2012). Diarrhoea is a clinical entity causes serious economic losses as it may lead to high mortality, weight loss or even late growth in different animals and even in human. It is caused by a combination of many risk factors (Bastawerous et al., 2001 and Gwyther et al., 2012).

The prevalence of E. coli O157:H7 can be high in some sheep ranches in California, especially in feedlots where young sheep are fed predominantly high-grain rations. (Kilonzo et al., 2012). These pathogens are responsible for great mortality and various morbidity changes and at the same time constitute a hazard to public health (Orden et al., 2000; Bolton et al., 2011 and Kilonzo et al., 2011).

Higgins et al. (2005) found that, diarrheogenic Escherichia coli, which include the enteropathogenic E. coli and the enterohaemorrhagric E. coli are a significant cause of diarrhoea I disease among infants and children in both developing and developed areas. There were four major categories of diarrhoeagenic E. coli, namely: entertoxygenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enteropathogenic E. coli (EPEC) and enterohaemorrhagic E. coli (EHEC). These categories of E. coli differ in their epidemiology and pathogenesis and their O : H serotypes (Mora et al., 2012).

A Shiga-toxin-producing Escherichia coli (STEC) strain belonging to serotype O104:H4, phylogenetic group B1 and sequence type ST678, with virulence features common to the enteroaggregative E. coli (EAEC) pathotype, was reported as the cause of the recent 2011 outbreak in Germany. The outbreak strain was determined to carry several virulence factors of extra-intestinal pathogenic E. coli (ExPEC) and to be resistant to a wide range of antibiotics. (Mora et al., 2012). Meat products may be contaminated with E.
coli from food handlers, utensils, air, soil and water as well as inadequate hygienic circumstance during manufacturing, packing and marketing of these products (Akanbi et al., 2011 and Wahl et al., 2011).

Polymerase chain reaction (PCR) technique has the advantage that it is readily applicable to large number of isolates, in contrast to classic methods such as agglutination, infant mouse, legated swine intestine and cell culture assays (Akanbi et al., 2011).

Evans et al. (2011) reported that, sheep have been proposed as a source of human verocytotoxigenic Escherichia coli infection on a number of occasions but few prevalence studies have focused on identifying rates of carriage of these pathogens in this species. The results showed that sheep presented for slaughter in Scotland may carry strains of E. coli, particularly of serogroups O157 and O26, which can be presumed to have potential to cause human infection. They did not support a hypothesis that human cases of E. coli O157:H7 are higher in any particular Scottish region as a direct consequence of a higher rate of faecal carriage in sheep in that region.

The components of virulence of Escherichia coli that include, protein toxins, many of which appear to play a role in disease process. These include alpha haemolysin, enterohaemolysin, verocytotoxins (Shiga-like toxins), cytotoxic necrotizing factor (CNF) and enterotoxins (Gyles, 1992). Also, Quinn et al. (2002) reported that the virulence factors of pathogenic strains of E. coli include capsule, endotoxin, structures responsible for colonization, enterotoxins and other secreted substances. The pathological effects of infection with pathogenic E. coli, other than attributed to endotoxin, derive mainly from the production of enterotoxins, verotoxins or cytotoxic necrotizing factors. Devenish et al. (1998) and Mersha et al. (2010) demonstrated that VT1, VT2 and the receptor-binding B subunit of VT1 all bind to non-glycolipid protein molecules on the surface of Vero cells which may act as potential receptors in addition to the Gb3 glycolipid receptor.

Tahamtan et al. (2010) reported that, Verocytotoxin producing Escherichia coli (VTEC) infection has been associated with diarrhoea. Sheep, like other ruminants, appear to have VTEC in their feces and are regarded as natural reservoirs of these pathogens. As contaminated sheep products can serve as a risk factor, their role as a food safety threat should be considered. The carcasses of sheep during slaughtering were examined for the presence of VTEC, which were isolated from 19 (9.5%) of 200 animals. Most of the 19 VTEC isolates (73.68%) contained Shiga toxin 1 and 2 genes. Eight (4%) carcasses of sheep were contaminated by E. coli O157:H7. The monthly prevalence of VTEC in sheep was obtained and ranged from 0.2% to 9.5% and was at its highest level in spring and late summer, which is in parallel to the seasonal variation in reported cases of O157 VTEC infections in humans. This study showed that VTEC are widely distributed in southern Iranian sheep.

Also, Escherichia coli produce haemolysin (Samer, 2001), also, it produce Cytotoxic Necrotizing Factor (CNF), the pathogenicity island termed locus of enterocyte effacement (LEE) encodes a type 3 protein secretion system, whose function is required for full virulence of enterohemorrhagic Escherichia coli (EHEC). The expression of ehxC fused with FLAG tag or a promoterless lacZ gene on pO157 was significantly induced under conditions in which GrlA was overproduced. These results together suggest that GrlA acts as a positive regulator for the ehx transcription in EHEC.
Garber et al. (1998) cited that samples containing *E. coli* isolates that expressed the O157 antigen and that were also positive for at least one verotoxin (SLT-I or SLT-II) were considered positive (VT-O157).

Ojo et al. (2010) cited that *E. coli* O157 is non-invasive, system sequelae must involve translocation of toxin from the gut lumen to underlying tissues. Damage to the intestinal epithelium by Shiga toxin, or other inflammatory mediators, may aid translocation of the toxin to the blood stream. (Beutin et al., 1989 and Law, 2000) found an association between Shiga toxin production and anovl haemolysin termed enterohaemolysin (Ehx).

This study was planned to determine the prevalence rate and virulence character and toxic production associated with *E. coli* from sheep.

**MATERIAL AND METHODS**

**Samples:** A total of 53 rectal swabs samples were collected from diarrhoeic sheep at different localities of Behera Governorate. The samples classified onto three groups. The first group consists of 40 sheep its aged from 1 to 6 months, the 2nd had 7 sheep aged from 7 to 12 month while the 3rd group had 6 sheep over one year old.

**Collection of samples:** Rectal swabs were taken from diarrhoeic sheep by means of sterile cotton swabs (Boyd et al., 1974). The collected samples were transferred in ice bags to the laboratory of the Department of Microbiology, Faculty of Veterinary Medicine Alexandria University. The samples were subjected to bacteriological examination as soon as possible.

**Cultivation of the samples for isolation of *E. coli* O157 : H7 (Kudra et al., 1997):**

Samples were incubated at 37 °C for 24 hours in trypticase soy broth supplemented with cefixin (50 µg/liter), potassium tellurite (2.5 mg/liter) and vancomycin (40 mg/liter). Aloopful of diluted broth were plated on to sorbitol MacConkey medium (SMAC) supplemented with cefixine and tellurite. Colonies were screened for sorbitol fermentation after overnight incubation at 37 °C.

**RESULTS AND DISCUSSION**

The results of incidence of *E. coli* isolated from fecal swabs of diarrhoeic sheep indicated that, the incidence of *E. coli* isolated in sheep differed significantly (P < 0.01) among examined sheep. The *E. coli* was isolated from 16 samples out from total 53 rectal swabs collected from diarrhoeic sheep with a percentage of (30.20 %). Our results agreed with those of Osman et al. (2012) where they isolated *E. coli* from diarrhoeic animals by 63.6% in calves, 27.3% in goat and 9.1% in sheep. The 102 *E. coli* strains isolated from the calves, goat and sheep were 100% haemolytic non-verotoxic and fitted into the age group.

The results of haemolytic activity of *E. coli* isolates showed that, all *E. coli* isolates haemolytic activity by a percentage of 100 %. Our results agreed with those of Wahl et al. (2011)
where they reported that, Escherichia coli (E. coli) O157:H7 can cause haemorrhagic colitis and the haemolytic uremic syndrome in humans. Ruminants are the main reservoir for this bacterium: they can harbour the bacteria in the gastrointestinal tract without showing clinical symptoms. The reason for this persistence is still unclear, although it has been suggested that E. coli O157:H7 can suppress the immune system. To investigate the effects on the immune system of ruminants, an infection model is needed that mimics a long-term infection as it can occur in both sheep and cattle. As the terminal rectum has recently been identified as a primary colonisation site in cattle, we developed a rectal inoculation model for sheep and used this model to study immune responses against selected virulence factors of E. coli O157:H7 ( intimin, EspA and EspB). Sheep were infected and re-infected when E. coli O157:H7 excretion was no longer detectable. The animals did not develop serum or local antibody responses but showed a cellular response against EspA and intimin respectively 9 and 16 days after infection. This response was also present 5 days after re-infection, albeit lower, and did not prevent animals from being re-infected. These results demonstrate that E. coli O157:H7 can be persistently present in the large intestine of sheep without inducing a clear protective immune response.

The results of tube agglutination test for the 16 E. coli O157 isolated by using E. coli H7 antiserum cleared that, the number of E. coli O157 were 1 isolates two isolates from them were +ve for H7 antiserum and the other was one –ve for H7 antiserum. Our results agreed with those of Narang et al. (2009) who reported that, Escherichia coli O157:H7 is a foodborne pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome. Positive identification of E. coli O157:H7 is made using biochemical tests and specific antisera or latex agglutination reagents for the O157 and H7 antigens. However, under certain conditions, some E. coli O157:H7 isolates can appear to be nonreactive with H7 antisera and may require multiple passages on motility medium to restore H7 antigenicity.

This study concluded that, infectious diseases of sheep are among the most notable constraints on the expansion of sheep production and the realization of its full potential. E. coli causes severe economic losses among sheep production farms and causes high mortalities; Coliforms are Gram-negative, rod-shaped facultatively anaerobic bacteria, the number of isolates differ significantly among different localities and sheep breeding
methods, the most important *E. coli* isolates, that isolated from positive sheep samples to *E. coli* were O157 : H7 and causes severe diarrhea and agglutination test is an a good method for identification of *E. coli* isolates.

Table (2): Incidence of *E. coli* isolated from diarrheic sheep:

<table>
<thead>
<tr>
<th>Animal species</th>
<th>No. of tested sample</th>
<th>No. of isolated <em>E. coli</em></th>
<th>Rate of isolation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>53</td>
<td>16</td>
<td>30.2</td>
</tr>
</tbody>
</table>

Chi² = 6.45**  ** = Significant at (P < 0.01)

Table (3): Results of *E. coli* serotyping.

<table>
<thead>
<tr>
<th>Total isolated <em>E. coli</em></th>
<th>E. coli O157</th>
<th>E. coli O157 : H7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Rates (%)</td>
</tr>
<tr>
<td>Sheep</td>
<td>1</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Chi² = 8.35**  ** = Significant at (P < 0.01)

REFERENCES


Microbiological Study of Escherichia coli in sheep

Edwards and Ewing (1972);


Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. C. and Leonard, C.

