ORIGINAL ARTICLES

Bacteriological studies on Escherichia coli producing verocytotoxin which cause diarrhea in sheep and goats in Saudi Arabia

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ABSTRACT

In this study, A total of 153 animals at Taif governorate were included (55 diseased sheep, 22 dead lambs, 42 diseased goats and 34 dead kids). The bacteriological examination revealed that *E. coli* was isolated from diarrheic sheep in percentage of 36.36%. Regarding to dead lambs *E. coli* was isolated from lung, liver, spleen and intestine in a percentage of 12 (54.55%), 15 (68.18%), 16 (72.73%), 18 (81.82%), respectively. *E. coli* was isolated from diarrheic goats in percentage of 35.71%. Regarding to dead kids *E. coli* was isolated from lung, liver, spleen and intestine in a percentage of 15 (44.12%), 20 (58.82%), 20 (58.82%), 21 (61.76%), respectively. Serological identification of isolated strains revealed that *E. coli* O128:H2, *E. coli* O146:H8 and *E. coli* O157:H7 were detected in percentage of 33 (44.60%), 28 (37.84%) and 8 (10.81%) respectively. ELISA using LPS as coating antigen revealed that of 69 serum samples collected from culture positive animals for *E. coli*, 67 (97.10%) were seropositive. From 79 serum samples obtained from culture negative animals, 7 (8.86%) were seropositives. From 69 tested *E. coli* isolates belonged to 3 serovars, 64 (92.75%) were positive for heamolysin test, 65 (94.20%) were able to survive in normal serum, 60 (86.96%) were MRHA and 9 (13.04%) were MSHA. All isolates 69 (100%) were positive for verocytotoxin production. All isolates of *E. coli* serovars were highly sensitive to Ampicillin, ciprofloxacin, ofloxacin and tobramycin (100% each) On the contrary all isolates were resistant to erythromycin. The use of *Lactobacillus acidophilus* induced inhibitory results on the growth of all isolated *E. coli serovars* in vitro. Eighty male Wister rats of 10-12 weeks old and weighing approximately 100 gm, the rats were divided into 8 equal groups (infected-with *E. coli* O128:H2 non treated [1], infected with *E. coli* O146:H8- non treated [2], infected with *E. coli* O157:H7- non treated [3], groups 4 & 5 infected with above mentioned serovars respectively and treated with *Lactobacillus acidophilus*. Group 7 administrated the *Lactobacillus acidophilus* only, group 8 kept as control group). Antibody titre measured by ELISA test in experimental animals increased from 1st week post infection in infected- treated groups (4 & 5) which more than that recorded in infected-non treated groups (1 & 2&3) This increase in antibody titre increase gradually till reach peak at 3 weeks post infection. Mortality rates in infected non treated groups (1 & 2&3 ) were 60%, 70% and 90% respectively while mortality rates among infected treated groups(4 & 5 ) were 0% each and was10% in group (6). Re-isolation of *E. coli serovars* from different organs of dead rats all over experimental period and sacrificed rats at the end of the experiment gave variable results. In infected-non treated groups (1 & 2&3) *E. coli* O128:H2 isolated from lungs, liver, spleen and intestine in a percent of 40%, 70%, 80% and100% respectively. While *E. coli* O146:H8 isolated in a percent of 20%, 50%, and 80% and100% respectively. *E. coli* O157:H7 isolated in a percent of 40%, 80%, 80% and100% respectively .In rats of infected-treated groups (4 & 5), *E. coli* O128:H2, *E. coli* O146:H8 couldn't be isolated from any internal organ *E. coli* O157:H7 isolated in a percent of 10% from spleen and 20% from intestine. The histopathological examination of naturally infected animals revealed that VTEC had a drastic severe pathological alteration represented by haemorrhagic pneumonia, haemorrhage and necrosis of hepatocytes, hemolytic-uremic syndrome as well as haemorrhagic colitis. The histopathological examination of rats groups (1 & 2&3) which died during the experiment showed nearly the same lesions as naturally infected animal. While the rats of the infected-treated groups (4 & 5) showed mild pathological changes such as mild necrosis and mild infiltration of mononuclear cells and their cells tend to be in normal state. These results confirmed the probiotic effect of *L. acidophilus* against the colonization of *E. coli* serovars in animal tissues as well as enhancing their immune response.

Key words: Bacteriological studies - Escherichia coli - verocytotoxin - diarrhea - Enteritis - diarrhea in sheep and goats

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Introduction

*E. coli* is one of the bacteria that exist in the normal microflora of the intestinal tract of humans and warm blooded animals. The enterohemorrhagic Escherichia coli (EHEC) and some other Shiga toxin-producing *E. coli* (STEC) strains are associated with disease in humans and animals (Kudva *et al.*, 1997).

Shiga toxin (Stx)-producing Escherichia coli (STEC) organisms, also called verocytotoxin (VT)-producing *E. coli* (VTEC), are one of the most important groups of food borne pathogens (Chapman *et al.*, 2000).

Infection can cause gastroenteritis that may be complicated by hemorrhagic colitis or the hemolytic-uremic syndrome (HUS) (Ulukanli and Çavli 2006).

Multiple virulence factors contribute to the pathogenicity of EHEC and include the production of a Shiga toxin (Stx) or Stxs, the ability to cause attaching-and-effacing lesions that disrupt microvilli on the intestinal wall of the host, and the possession of large plasmids encoding adhesins and hemolysins (Tesh, and O’Brien. 1992).

Enterohaemorrhagic strains of *E. coli* have two unique characteristics: they can produce toxins and colonize the intestinal tract of susceptible individuals. STEC are defined by the production of one or more types of Shiga toxin (Stx1 or Stx2 or their variants), which inhibit the protein synthesis of host cells, leading to cell death. Stx1 and Stx2 are encoded by alleles in the genome of temperate, lambdoid bacteriophages that are integrated in the *E. coli* chromosome (Islam, *et al.*, 2008).

Members of the Stx family are cytotoxins grouped according to both biological activity and antigenic characteristics to two distinct groups known as Stx1and Stx2. They are compound toxins, comprising a single catalytic 32-kDa A subunit and a multimeric B subunit (7.7-kDa monomers) that is involved in the binding of the toxin to specific glycolipid receptors present on the surface of target cells. A single EHEC strain may express Stx1 only, Stx2 only, or both toxins, or even multiple forms of Stx2 (Islam, *et al.*, 2008).

Many EHEC strains can produce intimin, an outer membrane protein involved in adhesion of bacteria to the intestinal mucosa. Intiminis a 97-kDa protein responsible for attachment. Production of enterohaemolysin has also been described as a virulence factor. EHEC-haemolysin (enterohaemolysin) is a pore-forming cytoxin, which is cytolytic to human and animal cells (Nweze, 2009).

Ruminants appear to be more frequently colonized by STEC than are other animals, but the reason(s) for this is unknown.

STEC is better adapted to persist in the alimentary tracts of sheep than are other pathotypes of *E. coli* (Cormick, *et al.*, 2000).

Conventional testing methods for verotoxin producing *E. coli* (VTEC) involve the isolation of the organism on culture from fecal samples followed by biochemical and immunologic confirmation. This method usually requires 72 h for complete identification.

Rapid, less labor-intensive methods are desirable (Dylla, 1995).

Lactobacillus species, normal microflora of digestive and urogenital tracts, have been reported to have antimicrobial activity in vitro, and when administered orally to poultry and mice and intravaginally to humans (Abd El-Moez *et al.*, 2008).

Using of probiotics help to protect the host from various intestinal diseases and disorders while increasing the number of beneficial bacteria and make the balance steady again. Probiotics are used for long times in food ingredients for human and also to feed the animals without any side effects. Also probiotics are acceptable because of being naturally in intestinal tract of healthy human and in foods (Çakır, 2003). Lactobacillus, which are known to adhere to the intestinal epithelial cells were providing a natural barrier and reducing pathogens’ adhesion ability. They are also known to increase immune function and to act as natural antibiotics to kill undesirable microbes (Salerno, 2011).

Therefore, to ascertain the level/spectrum of bacterial pathogens and to define the association of verotoxin producing *E. coli (VTEC)* with disease in sheep and goats in Saudia Arabia, a controlled study using the traditional culture/serology technique, identification of specific virulence factors, the antimicrobial susceptibility pattern of *Escherichia coli* as well as the specifically an antimicrobial effect of lactobacilli on isolated strains were undertaken.

Material and Methods

Field samples:

A total of 153 animals were included in this study (55 diseased sheep, 22 dead lambs, 42 diseased goats and 34 dead kids)
Fecal samples:

were collected from 55 diseased sheep and 42 diseased goats (older than 12 month) with which severe watery diarrhea, which may become grossly bloody, were associated. While internal organ samples (lung, liver, spleen and intestine) were collected from 22 dead lambs and 34 dead kids (less than 3 month old) which had a history of haemorrhagic colitis with bloody diarrhoea from different localities in Taif governorate. The samples were collected in a period from April to June 2011. Each collected fecal samples were divided into 3 parts, the first part were transferred rapidly on ice box to the laboratory for bacteriological examinations. The second and third parts were examined for any virological and parasitological pathogen.

While each collected tissue samples were divided into 4 parts, the first part were transferred rapidly on ice box to the laboratory for bacteriological examinations for histopathological studies. The second and forth parts were examined for any virological and parasitological pathogen.

Serum samples:

blood samples were collected from the same examined animals, and then the sera were separated and kept at -20°C in sterile bottles till examined.

Isolation and identification of E. coli:

It was carried out on the basis of Quinn et al., (2002). Ten grams from each samples were inoculated into nutrient broth and incubated aerobically at 37 °C for 24 h. Then a loopful from the inoculated broth were streaked onto plates of blood agar, MaCconkey’s bile salt agar and sorbitol MaCconkey agar (Difco Laboratories, Detroit, Mich.) supplemented with cefixime (0.05 mg/liter), potassium tellurite (2.5 mg/liter).

Suspected purified isolates were identified biochemically and were tested for agglutination with specific E.coli antisera (Pro- Lab Diagnostics, Richmond Hill, Ontario, Canada, and Difco Laboratories) according to Sanderson et al., (1995).

Isolation and identification of lactobacillus acidophilus:

lactobacillus acidophilus was isolated from the intestine of healthy calves on Rogosa agar medium at 37°C and 10% CO2 Isolation and identification was carried out according to Qin et al., (1995).

Humoral Immune Responses to Escherichia coli Lipopolysaccharide (LPS) in Examined animal sera:

Lipopolysaccharide was prepared from isolated E.coli strains by hot-phenol extraction according to Chart et al., (1989).

ELISA:

ELISA tests were performed based on procedures described previously by Chart et al., (1991).

Virulence factors testes:

1- Haemolysin production: Hemolysin production was assessed in nutrient agar with 5% washed horse erythrocytes a hemolytic zone larger than the overlying colony after overnight incubation was considered positive Hughes et al., (1983).

2- Serum resistance: was done according to method reported by Seigfried et al., (1994).

3-Receptor-binding properties: was determined according to Leffler and Svanborg-Edén, (1981).

4-Toxin production:

Toxin production was determined by Vero cell culture and was evaluated for toxic activity as described by Janke, et al., (2004).

In vitro antibiotic sensitivity assay:

Antibiotic sensitivity test of isolates was applied using disc diffusion technique according to Finegold and Martin (1982), and sub-culture of the isolates Results were interpreted according to the manufacture company (Oxoid).
**Lactobacillus acidophilus as probiotic, well diffusion assay:**

Selected cultures from the present bacterial isolates were plated on Muller Hinton agar plates and wells were drilled out using pasture pipettes. 50mL aliquots of cell free cultures supernatant in fresh M.R.S broth suspended in the agar wells. Plates were incubated for 48 to 72 hours under microaerophilic conditions at 37°C and the diameters of inhibition zones around wells were measured. Results were expressed as a mean diameter and standard error (Sgouras et al, 2004).

**Experimental Design:**

Eighty male Wister rats of 10-12 weeks old and weighing approximately 100 gm, were divided into 8 equal groups. The rats were kept for 2 weeks before the beginning of the experiment for adaptation and also for making sure that they free from any pathogen.

Group (1): Ten rats were fed for seven days on 10% skim milk only (Infected untreated group) before challenged with *E. coli* O128:H2 at a dose 1x10^9 CFU/ml intra-peritoneal injection (I/P).

Group (2): Ten rats were fed for seven days on 10% skim milk only (Infected untreated group) before challenged with *E. coli* O146:H8 at a dose 1x10^9 CFU/ml I/P.

Group (3): Ten rats were fed for seven days on 10% skim milk only (Infected untreated group) before challenged with *E. coli* O157:H7 at a dose 1x10^9 CFU/ml I/P.

Group (4): Ten rats were challenged with 1x10^9 CFU/ml *E. coli* O128:H2 I/P and simultaneously feed for seven days on 10% skim milk containing lactobacillus acidophilus1.5 x10^8 CFU/ml (Infected-treated group).

Group (5): Ten rats were challenged with 1x10^9 CFU/ml *E. coli* O146:H8 I/P and simultaneously feed for seven days on 10% skim milk containing lactobacillus acidophilus1.5 x10^8 CFU/ml (Infected-treated group).

Group (6): Ten rats were challenged with 1x10^9 CFU/ml *E. coli* O157:H7 I/P and simultaneously feed for seven days on 10% skim milk containing lactobacillus acidophilus1.5 x10^8 CFU/ml (Infected-treated group).

Group (7): Ten rats were administrated the lactobacillus acidophilus1.5 x10^8 CFU/ml only.

Group (8): Ten rats were kept as control group.

All animals were given standard chow diet and drinking water ad-libitum.

**Mortality rates:**

Mortality rates calculated as numbers of dead rats allover the experimental period in relation to all inoculated rats in each group.

**Samples collected from experimental animals:**

- Blood samples were collected from all rats each group 1, 2, 3 and 4 weeks post-challenge of *E. coli*. The serum was separated by centrifugation and stored at -20°C until used for ELISA testing.
- Tissues specimens were collected from all animals (lung, liver, spleen and intestine) and subjected to bacteriological examination for isolation and identification of *E. coli* serovars. Histopathological examination also was done on these collected tissues.

**ELISA test:**

Serum immunoglobulin G (IgG) antibodies titres were determined by an enzyme-linked immunosorbent assays as previously mentioned according to method of Chart et al, (1991), except 100 µl of rabbit antirat IgG peroxidase conjugate diluted 1:1000 in BPS containing 1% BSA were used instead of peroxidase-Labeled rabbit anti-sheep immunoglobulin G (IgG) diluted 1/10000 in PBS-T

**Bacteriological examination of experimental tissues:**

Lung, liver, spleen and intestine of experimental animals were collected under aseptic condition. Re-isolation of *E. coli* serovars was done as previously mentioned according to method of Kudva et al, (1997).

**Histopathological studies:**

Tissue specimens collected from both naturally infected animals and experimental rats groups were preserved in 10% neutral buffered formalin, and then processed to obtain five micron thick paraffin sections to be stained with haematoxylin and eosin according to Bancroft et al, 1996 for histopathological examination.
Results:

The clinical symptoms appeared on the examined animals were represented by abdominal cramps, bloody diarrhoea with low grade of fever.

The virological and parasitological examinations revealed negative results. The bacteriological examination revealed that E. coli was isolated from 20 diarrheic sheep out of 55 examined sheep in percentage of 36.36% (table, 1). Regarding to dead lambs E. coli was isolated from lung, liver, spleen and intestine in a percentage of 12 (54.55 %), 15 (68.18 %), 16 (72.73 %), 18 (81.82 %), respectively.

E. coli was isolated from 15 diarrheic goats out of 42 examined goats in percentage of 35.71% (table, 1). Regarding to dead kids E. coli was isolated from lung, liver, spleen and intestine in a percentage of 15(44.12 %), 20 (58.82 %), 20 (58.82 %), 21 (61.76 %), respectively.

E. coli O128:H2 was detected in 33 animals (44.60%), E. coli O146:H8 was detected in 28 animals (37.84%), whereas E. coli O157:H7 was detected in 8 animals (10.81%) as shown in table (2).

ELISA using LPS extracted from E. coli serovars as coating antigen has been used for detection of E. coli antibodies in examined animals as shown in table (3).

From 69 serum samples of E. coli culture positive animals 67 (97.10 %) were seropositive using LPS extracted from E. coli serovars using optical density, 0.745 respectively.

Concerning 79 serum samples obtained from culture negative animals, 7 (8.86%) were seropositives using LPS extracted from E. coli serovars.

From 69 tested E. coli isolates belonged to 3 serovars, 64 isolates with an incidence of 92.75% were positive for haemolysin test,65 with an incidence of 94.20% were able to survive in normal calf serum, 60 isolates with an incidence of 86.96% were MRHA and 9 isolates with an incidence of 13.04% were MSHA positive for verocytotoxine production (Fig. 1 & 2).

As shown in table 5 all isolates of E. coli serovars were highly sensitive to Ampicillin, ciprofloxacin, ofloxacin and tobramycin (100% each) On the contrary all isolates were resistant to erythromycin.

Gentamicin, oxytetracycline and trimethoprim + sulphamethoxazole, gave variable results.

The in vitro sensitivity of 69 E. coli recovered from examined animals to 8 antimicrobial agents was determined.

The use of Lactobacillus acidophilus (Table, 6) induced inhibitory results on the growth of all isolated E. coli serovars.

Antibody response measured by ELISA test in experimental animal groups was illustrated in table (7) which showed that an increase in the antibody titers from 1st week post infection in infected-tREATED groups (4 & 5)&6 ) which more than that recorded in infected-non treated groups (1 & 2&3 ) This increase in antibody titre increase gradually till reach peak at 3 weeks post infection.

Mortality rate (Table 8) in infected rats with E. coli O128:H2, E. coli O146:H8 and E. coli O157:H7 non treated groups were (1 & 2&3 ) 60%, 70%and 90% respectively while mortality rates among infected treated groups (4 & 5 ) were 0 % each and 10% in group (6).

Resisolation of E. coli serovars from different organs of dead rats all over experimental period and sacrificed rats at the end of the experiment gave variable results. In infected-non treated groups (1 & 2&3 ) E. coli O128:H2 isolated from lungs, liver, spleen and intestine in a percent of 40%, 70%, 80% and100% respectively.

While E. coli O146:H8 isolated in a percent of 20%, 50%, 80% and100% respectively. O157:H7 isolated in a percent of 40%, 80%, 80% and100% respectively .In rats of infected-treated groups (4 & 5), E. coli O128:H2, E. coli O146:H8 couldn't be isolated from any internal organ E. coli O157:H7 isolated in a percent of 10% from spleen and 20% from intestine, as shown in table 9.

Pathological results:

Gross findings of naturally infected animals showed congested and enlarged lungs, liver, kidneys, spleen and intestine, with bloody diarrhea in its lumen.

Histopathological examination of lungs showed haemorrhage filled the alveoli accompanied with lymphocytic infiltration (Haemorrhagic pneumonia) as well as destruction and sloughing of the epithelial cells lining the bronchioles, complete destruction of the endothelial cells lining the blood vessels with thrombus formation (Fig. 3). Liver showed haemorrhage, infiltration of mononuclear inflammatory cells, fibrinotic deposition especially around the portal area with necrosis of some hepatocytes (Fig. 4). Kidneys showed haemorrhage, necrosis of epithelial cells lining some renal tubules, infiltration of mononuclear inflammatory cell as well as vacuolation and necrosis of the endothelial cells lining the blood vessels of the glomeruli accompanied with infiltration of mononuclear inflammatory cells inside the Bowman's capsule (Fig. 5). Spleen was suffered from severe depletion of the lymphocytes of the white bulb as well as accumulation of hemosidrin pigments scattered in the red bulb (indication of haemorrhage) (Fig. 6). Intestine especially the right colon was...
the most severely affected part which showed severe haemorrhage, oedema, atrophied villi, fibrinous to fibrinohaemorrhagic exudates were fill the intestinal lumen (Fibrinohaemorrhagic colitis), necrosis of the epithelial cells lining the intestinal glands with sever infiltration of mononuclear inflammatory cells (Fig. 7).

The infected-non treated rats were suffered from abdominal pain and bloody diarrhoea which ended by death. Some rats can survived till the end of the experiment, had the same symptoms with non bloody diarrhoea.

The gross examination of dead rats of infected-non treated group showed congested, enlarged internal organs as well as oedematous intestinal wall and the intestinal lumen had mucofibrinous exudates tinged with blood. Otherwise the rats of the same group which can survive till the end of the experiment showed enlarged internal organs with non-bloody diarrhea in the intestinal lumen. While rats of other groups didn't show any gross lesions.

Histopathological findings of dead rats of the infected-non treated group were showed nearly the same lesions in all organs as the naturally infected animals. Lungs showed thrombus of small blood vessel, fibrous connective tissues around the bronchioles and the blood vessel, hyperproliferation of the cells lining the bronchioles as well as thickening of the wall of the blood vessel accompanied with infiltration of mononuclear inflammatory cells (Fig. 8). Liver was suffered from severe oedema with focal necrosis of hepatocytes and infiltration of inflammatory cells (Fig. 9). Kidneys showed vacuolation of glomerular tuft, necrosis of epithelial cells lining most of renal tubules with mononuclear inflammatory cells (Fig. 10). Spleen was suffered from depletion of the lymphocytes of the white bulb (Fig. 11). Some animals showed increase in the goblet cells of the intestinal villi with oedema and infiltration of inflammatory cells in-between the intestinal glands with fibrinotic exudates tinged with inflammatory cells in its lumen (Fig. 12). Complete destruction of the villi and also the intestinal gland in the payer's patch part of the intestine which engorged with lymphocytes (Fig. 13).

The histopathological examination of rats of infected-treated groups (4,5 and 6)were represented by mild oedema, few necrotic hepatocytes and very mild infiltration of mononuclear inflammatory cells in liver (Fig. 14). Kidneys showed normal glomeruli with few lymphocytes cell infiltration (Fig. 15). Spleen showed very mild depletion of lymphocytic cells of white bulb (Fig. 16). Intestine showed few inflammatory cells in the lumen of the villi (Fig. 17).

Rats of non-infected-treated rats didn't show any pathological alterations (group 7).

Fig. 1: Vero cell showing no alteration (negative control).

Fig. 2: Vero cell cytotoxicity indicated by cell rounding, enlargement and presence of several filamentous tendrils.
Fig. 3: Lung of naturally infected animals with E. coli showing haemorrhagic pneumonia and complete destruction of the endothelial cells lining the blood vessels with thrombus formation (H & E, X 200).

Fig. 4: Liver of naturally infected animals with E. coli showing haemorrhage, infiltration of mononuclear inflammatory cells, fibrinotic deposition specially around the portal area with necrosis of some hepatocytes (H & E, X 400).

Fig. 5: Kidney of naturally infected animals with E. coli showing haemorrhage, necrosis of epithelial cells lining some renal tubules, infiltration of mononuclear inflammatory cell as well as vacuolation and necrosis of the endothelial cells lining the blood vessels of the glomeruli (H & E, X 200).

Fig. 6: Spleen of naturally infected animals with E. coli showing severe depletion of the lymphocytes of the white bulb as well as accumulation of hemosidrin pigments scattered in the red bulb (H & E, X 200).
Fig. 7: Intestine of naturally infected animals with E. coli showing fibrinoheamorrhagic colitis and necrosis of the epithelial cells lining the intestinal glands with severe infiltration of mononuclear inflammatory cells (H & E, X 100).

Fig. 8: Lung of infected rats with E. coli showing thrombus of small blood vessel, fibrous connective tissues around the bronchioles and the blood vessel, hyperproliferation of the cells lining the bronchioles, thickening of wall of the blood vessel accompanied with infiltration of mononuclear inflammatory cells (H & E, X 200).

Fig. 9: Liver of infected rats with E. coli showing severe edema with focal necrosis of hepatocytes and infiltration of inflammatory cells (H & E, X 200).

Fig. 10: Kidney of infected rats with E. coli showing vacoulation of glomerular tuft, necrosis of epithelial cells lining most of renal tubules with mononuclear inflammatory cells (H & E, X 200).
Fig. 11: Spleen of infected rats with E. coli showing depletion of the lymphocytes of the white bulb (H & E, X 200)

Fig. 12: Intestine of infected rats with E. coli showing increase in the goblet cells of the intestinal villi with edema and infiltration of inflammatory cells in-between the intestinal glands with fibrinotic exudates tinged with inflammatory cells in its lumen (H & E, X 200).

Fig. 13: Intestine of infected rats with E. coli showing complete destruction of the villi and also the intestinal gland in the pyre's patch part of the intestine which engorged with lymphocytes (H & E, X 200)
Fig. 14: Liver of infected –treated groups by garlic powder showing mild edema, few necrotic hepatocytes and very mild infiltration of mononuclear inflammatory cells (H & E, X 200).

Fig. 15: Kidney of infected –treated groups showing normal glomeruli with few lymphocytes cell infiltration (H & E, X 100).

Fig. 16: Spleen of infected –treated group showing very mild depletion of lymphocytic cells of white bulb (H & E, X 100).
Fig. 17: Intestine of infected – traded group showing few inflammatory cells in the lumen of the villi (H & E, X 200).

Table 1: Isolation of sorbitol and non-sorbitol fermenting E. coli from diarrheic and dead lambs.

<table>
<thead>
<tr>
<th>Examined lambs</th>
<th>Types of samples</th>
<th>No. of examined samples</th>
<th>No. (%) of E. coli isolation *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrheic sheep (55)</td>
<td>Fecal</td>
<td>55</td>
<td>20 (36.36%)</td>
</tr>
<tr>
<td>Dead lambs (22)</td>
<td>Lungs</td>
<td>22</td>
<td>12 (54.55%)</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>22</td>
<td>15 (68.18%)</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>22</td>
<td>16 (72.73%)</td>
</tr>
<tr>
<td></td>
<td>Intestine</td>
<td>22</td>
<td>18 (81.82%)</td>
</tr>
<tr>
<td>Diarrheic goats (42)</td>
<td>Fecal</td>
<td>42</td>
<td>15 (35.71%)</td>
</tr>
<tr>
<td>Dead kids (34)</td>
<td>Lungs</td>
<td>34</td>
<td>15 (44.12%)</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>34</td>
<td>20 (58.82%)</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>34</td>
<td>20 (58.82%)</td>
</tr>
<tr>
<td></td>
<td>Intestine</td>
<td>34</td>
<td>21 (61.76%)</td>
</tr>
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</table>

* Percentage was calculated according to number of examined animals

Table 2: Serotypes of verocytotxin E. coli isolated from diarrheic and dead animals.

<table>
<thead>
<tr>
<th>E. coli Serovars</th>
<th>Diarrheic lambs</th>
<th>Dead lambs</th>
<th>Diarrheic goats</th>
<th>Dead kids</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%*</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>O128:H2</td>
<td>33</td>
<td>31</td>
<td>93</td>
<td>90%</td>
<td>33</td>
</tr>
<tr>
<td>O146:H8</td>
<td>28</td>
<td>28</td>
<td>100</td>
<td>100%</td>
<td>28</td>
</tr>
<tr>
<td>O157:H7</td>
<td>8</td>
<td>8</td>
<td>100</td>
<td>100%</td>
<td>8</td>
</tr>
<tr>
<td>Untypable E. coli</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>67</td>
<td>97</td>
<td>94%</td>
<td>69</td>
</tr>
</tbody>
</table>

* Percentage was calculated according to total number of isolates (74 isolates).

Table 3: Detection of Lps of E. coli antibodies in the serum of examined animals regarding to culture status

| Culture status Positive for | NO. | ELISA test using E. coli serovars LPS |
|                            |     | NO. | %   | optical density |
|                            |     |     |     |                |
| O128:H2                    | 33  | 31  | 93.94| 0.821          |
| O146:H8                    | 28  | 28  | 100  | 0.694          |
| O157:H7                    | 8   | 8   | 100  | 0.721          |
| Total positive             | 69  | 67  | 97.10| 0.745          |
| Untypable E. coli          | 5   | ND  | ND   | ND             |
| Total negative             | 79  | 8.86| 0.622|

Optical density ≥ 0.2 consider positive * = Mean optical density of positive animals

Table 4: Virulence characteristics of verocytotxin E. coli serovars isolated from examined animals.

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>No. tested strains</th>
<th>Haemolysin production</th>
<th>Serum resistance</th>
<th>Receptor-binding properties</th>
<th>Verocytotoxin production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>O128:H2</td>
<td>33 90.91%</td>
<td>29 87.88%</td>
<td>31 93.94%</td>
<td>2 6.06%</td>
<td>33 100%</td>
</tr>
<tr>
<td>O146:H8</td>
<td>28 92.86%</td>
<td>28 84.59%</td>
<td>22 66.67%</td>
<td>6 18.18%</td>
<td>28 100%</td>
</tr>
<tr>
<td>O157:H7</td>
<td>8 100%</td>
<td>8 100%</td>
<td>7 87.50%</td>
<td>1 12.5%</td>
<td>8 100%</td>
</tr>
<tr>
<td>Total</td>
<td>69 92.75%</td>
<td>66 94.20%</td>
<td>9 13.04%</td>
<td>69 100%</td>
<td></td>
</tr>
</tbody>
</table>

Optical density ≥ 0.2 consider positive
### Table 5: Results of antimicrobial sensitivity of 69 verocytotoxine E. coli serovars recovered from examined animal

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Disk conc. (µg/ml)</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 58 84.05</td>
<td>11 15.94</td>
<td>0 0.00</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 69 100</td>
<td>0 0.00</td>
<td>0 0.00</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 0 0.00</td>
<td>0 0.00</td>
<td>69 100</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 41 59.42</td>
<td>28 40.58</td>
<td>0 0.00</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>5 69 100</td>
<td>0 0.00</td>
<td>0 0.00</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0 0.00</td>
<td>26 37.68</td>
<td>43 62.32</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>10 69 100</td>
<td>0 0.00</td>
<td>0 0.00</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim+ sulphamethoxazole</td>
<td>1.25+23.75</td>
<td>0 0.00</td>
<td>21 30.43</td>
<td>48 69.57</td>
</tr>
</tbody>
</table>

S = Sensitive  I = Intermediate  R = Resistant

% calculated according to no. of examined isolates (69).

### Table 6: Antibacterial effect of probiotic bacteria on E. coli serovars in vivo.

<table>
<thead>
<tr>
<th>Tested strains</th>
<th>NO. of tested strains</th>
<th>Sensitive strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>O128:H2</td>
<td>33</td>
<td>33 100%</td>
</tr>
<tr>
<td>O146:H8</td>
<td>28</td>
<td>28 100%</td>
</tr>
<tr>
<td>O157:H7</td>
<td>8</td>
<td>8 100%</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>69 100%</td>
</tr>
</tbody>
</table>

### Table 7: Overall mean of ELISA optical density among experimental animal groups.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>NO. of dead rats/week post challenging</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>6/10 60%</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>9/10 90%</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/10 0%</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/10 0%</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/10 0%</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/10 0%</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/10 0%</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/10 0%</td>
</tr>
</tbody>
</table>

Optical density ≥ 0.2 consider positive*= Mean optical density of positive animals

### Table 8: Protective immunity induced by probiotic bacteria challenged by E. coli serovars

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>No. of dead rats/week post challenging</th>
<th>Dead/Total</th>
<th>Mortality rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>6 10/10</td>
<td>10/10 10%</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>7 10/10</td>
<td>10/10 10%</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>6 10/10</td>
<td>10/10 10%</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>6 10/10</td>
<td>10/10 10%</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>6 10/10</td>
<td>10/10 10%</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>6 10/10</td>
<td>10/10 10%</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>6 10/10</td>
<td>10/10 10%</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>6 10/10</td>
<td>10/10 10%</td>
</tr>
</tbody>
</table>

### Table 9: Re-isolation of E. coli serovars from different organs of dead as well as sacrificed rats during and at the end of the experiment.

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Lungs</th>
<th>Liver</th>
<th>Spleen</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4/10  (40%)</td>
<td>7/10 (70%)</td>
<td>8/10 (80%)</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>2</td>
<td>2/10 (0%)</td>
<td>5/10 (0%)</td>
<td>8/10 (0%)</td>
<td>10/10 (0%)</td>
</tr>
<tr>
<td>3</td>
<td>4/10 (0%)</td>
<td>8/10 (0%)</td>
<td>8/10 (0%)</td>
<td>10/10 (0%)</td>
</tr>
<tr>
<td>4</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>5</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>6</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>1/10 (0%)</td>
<td>2/10 (0%)</td>
</tr>
<tr>
<td>7</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>8</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
</tr>
</tbody>
</table>
Discussion:

Sheep and goats have been subjected to fewer epidemiological surveys than cattle. Recently, *E. coli O157:H7* has been detected in sheep and goats (Mainil, 1999). This suggests that small ruminants also may represent a source of transmission of VTEC to humans. Fecal samples were collected from 55 diseased sheep and 42 diseased goats (older than 6 months) with which severe watery diarrhea, which may become grossly bloody. While internal organ samples (lung, liver, spleen and intestine) were collected from 22 dead lambs and 34 dead kids (less than 3 month old) which had a history of haemorrhagic colitis with bloody diarrhoea from different localities in El-Taef governorate. *E. coli O157:H7* was isolated from eight (10.81%) of total number of isolates, whereas non-O157 VTEC as *O128:H2* and *O146:H8* were isolated in an incidence of 44.60% and 37.84% of total number of isolates respectively. These results nearly coincided with Beutin *et al.*, (1993). who found that the Prevalence rates of non-O157 VTEC were 67% in sheep and 56% goats. Kudva *et al.*, (1997) detected VTEC strains of 43% of sheep in the United States.


This study provides the first evidence that the prevalence of VTEC were higher in small animals (81.82% in lambs 61.76% in kids) than large animals (36.36% in sheep 35.71% in goats). These results may attribute to immune status of these small animals and/or sloppy environmental conditions and poor sanitation. (Beutin *et al.*, 1993).

The emergences of VTEC as a significant animal pathogen and its recognition as a zoonotic agent have stimulated research on technique for detection of infected herds.

Bacterial culture can be used to establish a diagnosis of VTEC but this method was time consuming and some time fails especially in animal treated with antibiotic. The detection of antibodies to VTEC LPS might overcome these problems inherent to this technique.

In the present study, LPSs antigen based indirect ELISA to detect antibodies against VTEC in sera of examined animals was developed and compared with culture results (Tables 3). Of 69 serum samples collected from culturally positive animals 67 (97.10%) were seropositive with LPSs antigens extracted from VTEC whereas from 79 serum samples collected from culturally negative animals 7 (8.85%) were also seropositive with the same antigens, respectively. This result is in accordance with Chart *et al.*, (1998). who reported that the ELISAs for IgM and IgG to *E. coli* 0157 LPS provided valuable and sensitive adjuncts to culture.

The antiibiogram of pathogens could be variable from place to place and from case to another. This may be explained by the wide use of chemotherapeutic drugs and the variation in its use which may produce new resistant mutants.

For this reason, one of the steps in the controlling of VTEC is the use of appropriate antibiotics. The kind of antibiotic should better be selected on the basis of its sensitivity which could be detected by laboratory examination.

Several virulence factors detected from pathogenic *E. coli* bacterium causing urinary tract infection, diarrhea, septicemia and meningitis.

The interesting objective goal in this work was to investigate the virulence attributes of *E. coli* which include: haemolysin production, serum resistance, haemaggululation test, and cytotoxine production. A total of 69 Escherichia coli isolates were selected from diseased and dead sheep and goats. These were investigated for their possession of virulence factors.

Haemolysin production was observed in 64 (92.75%) of isolated strains. Looking on pathogenic potential of *E. coli*, the most important trait as presented by Paton and Paton (1998), is the capability of *E. coli* strains to produce enterohaemolysin.

Some authors (Natoro and Kaper, 1998 and Schmidt *et al.*, 1999), also judged a role of haemolysin that is well-known to take part in pathogenesis of *E. coli* infections.

Out of 69 isolates of *E. coli* recovered from examined animals, 65 (94.20%) were able to survive in normal serum.

In this aspect, Rizvi and Kumar 2003 showed that the susceptibility of the organisms to the bactericidal action of normal serum is related to virulence in *E. coli*. Virulent strains were able to survive in fresh normal sera, whereas their avirulent variants were readily killed.

Agglutination of erythrocyte is an indirect evidence of the presence of fimbriae and it provides a simple indirect method of virulence testing. Examination of the recovered *E. coli* isolates for haemaggulination was done as shown in Tables (4). Among the isolates recovered from examined animals 86.96% and 13.04% were positive for MRHA and MSHA, respectively using guinea pig RBCs. These results agree with that of Old (1985), who used guinea pig RBCs as indicator for haemaggulination activity. Many bacteria produce surface antigens which enable adherence to epithelial cells in vivo and may promote agglutination of different species of erythrocytes (RBCs) in vitro. Such adherence factors are in many cases encoded by plasmids (Schmidt and Karch, 1996). The ability of *E. coli* isolates to agglutinate guinea pig RBCs in vitro was displayed only by...
bacteria having the fibrillae exposed on their surface, non fibrillated strains were invariably negative (Presterl et al., 2003).

Haemagglutinins (HA) was correlated with the presence of distinct type of fibrillae of 8 nm diameter. It is not known, however, whether these fibrillae are produced in vivo by haemagglutinating strains of E. coli, or whether they aid colonization of the intestinal surface (Elías et al., 1999).

According to cytotoxicity of tested E. coli strains from table (4) photo (1,2), all E. coli isolates obtained from examined animals produced distinct cytopathic response for Vero cells suggesting the production of cytotoxins.

The VTEC are capable of producing one or two potent toxins called verotoxins (VT1 and VT2) that have cytotoxic effects on African Green Monkey kidney (Vero) cells in cultures. In addition to toxin production, another virulence-associated factor expressed by VTEC is a protein called intimin, which is responsible for intimate attachment of VTEC to the intestinal epithelial cells, causing attaching and effacing lesions in the intestinal mucosa (Blanco et al., 2001).

The aforementioned recovered isolates of VTEC were tested for their sensitivity to 8 different antimicrobial agents. Results are shown in tables 5.

As shown in table 5, the most effective antibiotics for VTEC were, Ciprofloxacin, Ofloxacin and Tobramycin (100% each).

The superiority of flumequine and Tobramycin for the in vitro action in was E. coli reported by Abd El-Mozej et al., (2008), who reported that E. coli isolates of animal origin were sensitive to flumequine and Tobramycin.

Also, Reisdal (2000) recorded that all tested strains of E. coli were highly sensitive to Tobramycin. Many authors ruled out the use of antibiotics and favored the use of probiotics. Lactobacillus exhibited antimicrobial activity against wide range of Gram positive and Gram –ve bacteria without mutagenicity (Reid et al., 2006). The bacteriological studies revealed that Lactobacillus had inhibitory effect on all examined E. coli serovare (Table6). (Kinet and Lucchini, 1999).

The obtained results in table 7 showed that daily administration of Lactobacillus enhance antibody levels in all infected-treated groups (4 & 5&6). This increase in antibody titre was elucidated by many workers (De Macias et al., 1993 and Nemcova et al., 1999) who confirmed the immune stimulating effect of Lactobacillus on the cell-mediated as well as the humeral immunity. They also noticed that the Lactobacillus had a strong adjuvancity and initiated the induction of lymphokines and immunoglobulines.

Mortality rate (Table 8) in infected rats with E. coli O128:H2, E. coli O146:H8 and E. coli O157:H7 non treated groups were (1 & 2&3) 60%, 70% and 90% respectively while mortality rates among infected treated groups (4 & 5) were 0 % each and 10% in group (6).

Reisolation of E. coli serovars from different organs of dead rats all over experimental period and sacrificed rats at the end of the experiment gave variable results. In infected-non treated groups (1 & 2&3) E. coli O128:H2 isolated from lungs, liver, spleen and intestine in a percent of 40%, 70%, 80% and 100% respectively. While E. coli O146:H8 isolated in a percent of 20%, 50%, and 80% and 100% respectively. O157:H7 isolated in a percent of 40%, 80%, 80% and 100% respectively. In rats of infected-treated groups (4 & 5), E. coli O128:H2, E. coli O146:H8 couldn’t be isolated from any internal organ. E. coli O157:H7 isolated in a percent of 10% from spleen and 20% from intestine. (Table, 9). These results elucidated the protective effect of Lactobacillus which encompassed cell-associated and cell–free components e.g. S-layer hydrophobic protein, polysaccharides and lipoteichoic acid polymers. These components enhanced he adherence of L.acidophilius to intestinal mucosa, and hindered the colonization of E. coli and other pathogens (Nemcova et al., 1999). Other L.acidophilius cell – free products were acetate, lactate,H2O2, butyric acid, butyrate as well as lactoperoxidase thiocynate system (LPT),all these components had an inhibitory effect on the E. coli (Roberfroid, 1998). The Lactobacillus – primed T- cells enhanced the formation of epithelioid granulomas in the liver and spleen which was accompanied by activation of migratory macrophages and monocytes (Fairbrother et al., 1998).This findings also explained the disappearance of challenge E.coli isolate from liver and spleen of the L.acidophilius protected animals.

The histopathological examination of either naturally infected animals or infected non treated rats which died during the experiment revealed severe haemorrhage, oedema, destruction of the endothelial cells lining the blood vessels with thrombus formation and infiltration of mononuclear inflammatory cells, i.e systemic haemorrhagic syndrome (haemolytic uremic syndrome and fibrohaemorrhagic colitis) which lead to the death of the infected animals. These results were coincided with those noticed by Baker et al., (2007) who attributed these lesions to the infection with VTEC. Payer's patches was the target seat of this microorganism. All these lesions may be attributed to the fact that the isolates of VTEC produced several factors which contribute to their virulence; Shiga like toxin (SLTs) 1, 2 and several proteins encoded in locus of enterocyte effacement pathogenicity mainly endothelial cells lining the blood vessels leading to vascular damage and haemorrhage (Baker et al., 2007 and Zotta et al., 2008). In addition, Nyström, (1997) mentioned that following bacterial colonization of the intestine, the toxins are thought to enter the systemic circulation SLTs were then transferred.
to endothelial cells and cause damage with fibrin deposition in, also SLTs appeared to be capable of causing direct damage to tissues other than the endothelial cells. While, Boyee et al., (1995) added that the vascular damage by SLTs may allow to lipopolysacharride and other inflammatory mediators to gain access of circulation and initiating the hemolytic-uremic syndrome. Baker et al., (2007) mentioned that lesions which appeared in naturally infected calves and lamb were similar to those found in experimentally infected animals with E. coli O157:H7.

Rats of infected-non treated group which slaughtered at the end of experiment may be attributed to individual host immune status (Giraud et al., 2005) so, they showed necrosis of epithelial cells of all examined organs with edema and infiltration of lymphocytes which reflect as depletion of the lymphocytes of white bulb of spleen. While, intestine showed all stages of sloughed, eroded and complete necrosis of the villi as well as the epithelial lining the intestinal glands. Moreover, the increase in the goblet cells as well as the diffusion of some villi together, acts as a defense trial from the body against the microorganism invasion and its toxin production. These results were come in agreement with Karch et al., (2005) who suggested that cooperation between SLTs and tumor necrosis factor (TNF) may be important in producing the pathologic changes. i.e. TNF and SLTs exhibited synergistic cytotoxic activity towards endothelial and epithelial cells of these organs. While, rats of the infected-treated groups showed mild pathological changes such as mild necrosis and mild infiltration of mononuclear cells and their cells tend to be in normal state. These results may be referred to the action of L.acidophilus as antibacterial against enteric bacteria as previously mentioned by Roberfroid (1998). Moreover L.acidophilus was found to exhibit antimicrobial activity against a wide range of Gram –ve and Gram +ve bacteria including multidrug-resistant enterotoxigenic strain of E. coli (Nemcova et al., 1999).

Probiotics have their therapeutic effect, in part, by their ability to attach to enterocytes. Probiotics may produce lactic acid, bacteriocins and antimicrobial peptides, which are active against pathogens. They also produce mucosal micronutrients, eliminate toxins and reduce fecal ammonia, which can be toxic to the intestinal mucosa. (Salerno 2011).

Fairbrother et al., (1998) restored to the use of probiotics and biological preparations of Lactobacilli and other lactic acid bacteria to combat enteric bacteria .Furthermore it was considered as one of the major health products and immune potentiators (Roberfroid 1998).

From this study, it is found that VTEC is virulence microorganisms caused severe drastic pathological lesions can lead to the death of animals either by naturally or experimentally infection. Rats is represented a good model of EHEC infection. Such potential probiotic effect of L.acidophilus indicated a realistic route for using such biological preparations in the prevention and control of VTEC.

Conclusion:

- This study provides the first evidence that the prevalence of VTEC were higher in small animals than large animals'. These results may attributed to immune status of these small animals and/or sloppy environmental conditions and poor sanitation.
- The ELISAs for IgM and IgG to E. coli 0157 LPS provided valuable and sensitive adjuncts to culture.
- The histopathological examination revealed that VTEC had a drastic severe pathological alteration represented by haemorrhagic pneumonia, haemorrhage and necrosis of hepatocytes, hemolytic-uremic syndrome as well as haemorrhagic colitis
- Generally, VTEV virulence is a complex and multifactorial phenomenon, no individual test was found as a single reliable measure of virulence. However, questions about the association between virulence factors and illness remain without answer.
- The antibiogram of pathogens could be variable from place to place and from case to another.
- The kind of antibiotic should better be selected on the basis of its sensitivity which could be detected by laboratory examination.
- L.acidophilus was found to exhibit antimicrobial activity against VTEC strains of E. coli. Furthermore it was considered as one of the major health products and immune potentiators

Recommendations to prevent animal infection and reduce the public health risk

The economic loss to the livestock industry due to VTEC, judging from the morbidity and mortality especially in young animals'. The public health consequences are also enormous. Animals spread the organism to people by direct contact and indirectly via droppings and manure on land. However Reasonable control measures, based on the existing social and health systems in the country are possible. Immediate interim control measures include:

- Separating young animals from adults shows some effect in reducing prevalence/shedding of E. coli sheep and goats
- Feed and water may serve as sources by which E. coli can enter the production unit. Water trough design is an important factor in the potential effectiveness of using water chlorination. Animals should be prevented from standing in or defecating in the troughs.
- Ensure that water from burns and streams is treated before drinking
- Remove animals from field for 3 weeks prior to use
- Keep animals off field during use
- Remove all obvious animal droppings at the beginning of the 3 week period
- During recreational use always wash hands before eating, drinking and smoking using soap, clean towels and preferably hot and cold running water
- Treatment of affected animals with appropriate antibiotics to reduce fecal shedding of E. coli and eliminate infection.
- Feeding hay for a brief period immediately before slaughter may reduce the risk of food-borne E. coli infection.
- Vaccines are being investigated that produce antibodies that would prevent adherence and colonization of E. coli O157:H7.
- Supplementing animals with certain probiotic cultures has been shown experimentally to eliminate or decrease the incidence of E. coli in the feces of animals

References


Lactobacillus casei strain Shirota. Applied Environmental Microbiology, 70: 518-526.