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BACTERIOLOGICAL AND IMMUNOLOGICAL STUDIES ON *ESCHERICHIA COLI* ISOLATES RECOVERED FROM DIARRHOEIC AND CONTACT APPARENTLY HEALTHY SHEEP WITH HISTOPATHOLOGICAL CHANGES ENCOUNTERED

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SUMMARY

Bacteriolological examination of organ specimens collected from 18 necropsied local breed sheep, that had history of diarrhoea revealed the isolation of *Escherichia coli* in 13/18 (72.2%) of the animals, whereas in the faecal samples from diarrhoeic and contact apparently healthy sheep the incidence of *E. coli* was 42/59 (71.2%) and 17/35 (48.6%) respectively. The identified 72 E. coli isolates were affiliated to 9 serogroups, O76 (13), O16 (10), O5 (10), O28 (8), O117 (7), O145 (5), O91 (3), O6 (3), O157 (3) and untypable (10). All serogroups adhered better on enterocytes cell-model than on HEp-2 cells, as 53/72 (73.6%) of the isolates adhered with an average of 63.5 ± 6.2 *E. coli* / enterocyte, whereas on HEp-2 cells the adhesion was significantly lower ($P<0.05$) as 45/72 (62.5%) of the isolates were adherent with an average of 53.9 ± 6.7 *E. coli*/ cell. In verotoxin-production assay, 41/72 (56%) of the isolates were verotoxin producer.The OMPs extract of O76, O16 and O5 (which were the most dominant serogroups) were similar and had dense peptide bands between the range of 35kDa to 42 kDa. Lighter peptide bands were also demonstrated in the higher and lower gel ends. Immunoblotting analysis, also showed strong immunogenic protein bands at 35-37kDa and 40-42kDa against sera from infected sheep as well as against sera from rabbits immunized with a pool from the extracted OMPs. Using sera from immunized rabbits as adhesion inhibitors in the previously mentioned cell models revealed significant inhibition ($P<0.01$) of the adhesion pattern on both previously mentioned cell models. The inhibition averaged 63.7% reduction in the adhesion capacity of enterocyte cell model (23.0 ± 5.7

E. coli/cell), whereas the inhibition of adhesion averaged 52.3% in HEp-2 cell-model (20.2 ± 3.4 *E. coli*/cell). Histopathological findings of necropsied sheep revealed intestine mucosa had coagulative necrosis associated with inflammatory cells infiltrations. Diffuse desquamations and erosions in the mucosa of the villi. Aggrigation of epithelioid and lymphoid cells was also demonstrated in the lamina propria. Lung, interstitial stroma was thickened; bronchioles and alveoli had focal epithelioid stratification, desquamations and ulcerations. Liver was infiltrated by neutrophils, macrophages, lymphocytes and Kupffer cell proliferation. Kidney showed coagulative necrosis of renal tubules. Heart and spleen sections showed also pathological changes which also confirmed the systemic infection with attaching and toxigenic *E. coli*. It was concluded that OMPs can be used as an effective, highly immunogenic vaccine for controlling infection with *E. coli* in sheep as well as other animals.

INTRODUCTION

Adhesion and toxin production are essential virulence criteria for induction of pathogeneses of attaching and effacing *E. coli* (AEEC) and enterotoxigenic *E. coli* (ETEC). Both groups continue to cause serious gastrointestinal infections in sheep and other animal species (McCluskey et al., 1999; Wani et al., 2003). The infection can be transmitted to humans causing serious illnesses through consumption of contaminated animal products. Sheep were considered among the major reservoirs of different pathogenic serogroups as, O6, O5, O91, O115, O117, O128, O76, O16, O75; O157, O146 and O166 (Cookson et al., 2002; Rey et al., 2003; Vettorato et al., 2003). The severity of the infection was always augmented when additional virulence factors were encountered (Zweifel and Stephan 2003). The ability of the *E. coli* to adhere in-vitro to different cell-model was used to determine its ability to adhere in vivo. Many trials were conducted to curb the *E. coli* infection by vaccination with highly immunogenic outer membrane proteins as OMP-A, Pho-E, Lam-B, Tra-T OMP 35-37KDa which were considered superior to the whole cell vaccines (Gyles 1994). The epitopes which were masked in the non-eluted whole-cell antigens were exposed in the case of OMPs, enhancing an augmented immune response. The elicited immune response was monitored by ELISA or Immunoblotting (Kawahara et al., 1994). Low molecular weight OMPs were detected as immunogens from enterohaemorrhagic verotoxin producing O157 and O26 (Zhao et al., 1996 and Wang et al., 2002). Another essential OMP (54KDa) was reported by Patel and Austen (1996). The pattern of these OMPs was heterogeneous in some serogroups, but in many others was homologous as in O1, O2, O78 (Chaffer et al., 1999) Histopathological investigation of the necropsied sheep infected with *E. coli* was confirmatory in the diagnosis. Severe diverse lesions were observed in the intestines, varied from necrosis, erosion, haemorrhages, gra-

nulomas and atrophy of intestinal villi. Other internal organs were also affected due to the effect of circulatory toxins (Martin and Aitken, 2000).

The objective of this study was to detect the different *E. coli* serogroups causing the infection in sheep flocks and the histopathological alterations encountered, to demonstrate the adherence capacity to different host cell pattern, including the outer membrane protein (OMP) which could be putative potential immunogen and utilized in vaccine production.

MATERIALS AND METHODS

Collection of samples

A total of 108 internal organ specimens (18 intestinal sections from illium and deudinum 18 livers, 18 lungs, 18 spleens, 18 kidneys, 18 hearts) collected from 18 necropsied local breed sheep, aged from 2 weeks to 3 month, that had history of severe diarrhoea and fever. Also 59 faecal samples collected from diarrhoeic and 35 contact apparently healthy sheep (Table 1). Blood samples were also collected from 10 diseased and 10 apparently healthy sheep for immunoblotting test. These specimens were collected from governmental and private sheep flocks in Giza and El-Menia governorates. All specimens were transferred to the laboratory with minimal delay in ice box for the bacteriological examination. For histopathological examination specimens were fixed in 10% neutral buffered formalin.

Isolation and Identification of *E. coli*.

Specimens were cultured directly on blood agar (10% sheep blood) and MacConkey agar plates, incubated at 37°C for 24h. Suspected colonies were confirmed morphologically biochemically by conventional methods. (Quinn et al., 1994). Isolates were further confirmed by API-20 diagnostic strips (Bio Merieux, France). Serological Identification of *E. coli* was carried out by slide agglutination test using polyvalent and monovalent somatic (O) and flagellar (H) antigens (Difco Laboratories USA) according to the method of (Ewing 1986).

Toxigenicity of *E. coli* isolates.

Toxigenicity of the isolates was tested by growing the bacteria into casamino acid-yeast extract fluid media for 12h. at 41°C. Cultures were centrifuged at 10,000xg for 10min. and supernatants, which contained the Vero toxins were filtered with millipore membrane filter (0.22µm). Then 100µl of the filtrate were added to confluent monolayer of Vero cells and evaluated for cytopathic effect (CPE) for 72h while incubated at 37°C in 5% CO_2 atmosphere (Vettorato et al., 2003).

Preparation of *E. coli* OMPs

OMPs were isolated by growing selected *E. coli* isolates overnight at 37°C in Luria broth. Cells were recovered by centrifugation (6,000xg for 10min at 48°C) and suspended in 3ml of HEPES (N-2-hydroxyethylpiperazine-N9-2-

ethanesulfonic acid10mM, pH 7.4) solution. They were disrupted by sonication (Branson Sonifier; 60s, 30% output). Cell debris was removed by centrifugation at 6,000xg for10min at 48°C. The supernatant was added to 0.75ml of 2% N-lauroylsarcosine (Sarkosyl) and incubated for 15min at room temperature. The OMP was separated after centrifugation of the mixture at 100,000xg for 1h. The pelleted protein was resuspended in 10 mM HEPES and stored at -20°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with a 4% stacking and an 8% separating gel, after OMP preparations were solubilized at 100°C for 5 min in sample solution (0.05MTris-HCl [pH 8.0], 2.5% SDS, 5% 2-mercaptoethanol, 25% glycerol, 0.003% bromophenol blue). The proteins were visualized after the gels were stained with Coomassie brilliant blue R250 and destained with 30% methanol in 10% acetic acid (Debroy et al., 1995). The protein content of these OMP preparations were then estimated by Bradford, (1976) (Chaffer et al., 1999).

Immune-blotting.

The proteins in the polyacrylamide gels were transferred to nitrocellulose filters (Trans Blot Transfer medium; Bio-Rad Laboratories, Richmond, Calif.) by transblotting, according to the protocol described by Coligan et al., (1992). The nonspecific antibody sites on the proteins captured on the nitrocellulose were blocked by incubating the filter with blocking solution (5% non fat dry milk powder in PBS) for 1h at room temperature. The electroblotted proteins were probed with the primary antibody. The OMP antiserum (50 ml) was diluted in 5 ml of blocking solution and incubated for 1 h at room temperature with constant agitation. The nonspecifically bound OMP antibodies were removed by washing four times with 200 ml of PBS each time. The nitrocellulose filter was incubated with a solution of horseradish peroxidase - anti - immunoglobulin conjugate (Sigma Chemical) diluted 100-fold in blocking buffer for 1h at room temperature. The bound antibody was detected by the color reaction developed by the addition of diaminobenzidine substrate solution (Sigma) (Hellman et al., 2000).

Production of antibodies.

For production of antibodies against OMPs, 3 rabbits were immunized by injecting 200 mg of the OMP preparations, isolated from O76, O16 and O5 subcutaneously twice at 30-day intervals, first in complete and then in incomplete Freundís adjuvant. Blood was collected from the central auricular vein of each ear and then from the cephalic vein of the left leg 15 days later. The blood clot was removed from the sample, and the serum was prepared by centrifuging the blood at 2,000 rpm and 4°C for 20 min. The serum was stored at -20°C Coligan et al., (1992).

Adhesion and adhesion inhibition of *E. coli* to enterocytes and Hep-2 cells

Adhesion assay was performed on two kinds of cells, namely ovine enterocytes (Gyles, 1994) and Hep-2 cells (Ruttler et al., 2002). Entrocytes were collected from small intestine of 7-10 day-freshly sacrificed lamb. Intestines were washed in 5mM phosphate buffer containing 5mM EDTA (pH 7.4). Mucosa was scrapped and suspended in EDTA buffer then disrupted in a tissue grinder. Cells were then centrifuged at 180xg for 20min and suspended in PBS (pH 6.8) at approximately $2x10^7$ cells/ml. *E. coli* were grown in Penassay broth (Difco) at 37°C for 18h, washed with PBS (pH 6.8) and suspended in PBS containing 1% D-mannose, at approximately $2x10^9$ cells. In round-bottom microtiter plates equal volumes of brush border suspension and *E. coil* suspension were mixed. Plates were incubated at 37°C for 20min, and adhesion was examined on 20 enterocytes on microscope slide after gentle washing with PBS ph 7.4. For Hep-2 cells, the adhesion was carried out according to Ruttler et al., (2002). In adhesion inhibition test the bacterial cells were previously incubated with the hyper immune serum prepared in rabbits against OMPs as described by Ruttler et al., (2002).

Histopathological examination

Post-mortem examination was performed on necropsied animals and tissue specimens were taken from different internal organs. The specimens were fixed in10% neutral buffered formalin for at least 24hrs, then washed in running water and dehydrated in alcohol, then cleared in xylol and embedded in paraffin wax. Tissue sections were prepared at 4-6 micron thickness and stained with haematoxylin and eosin (Bancroft et al., 1994).

Statistical analysis

Statistical analysis of the obtained results was carried out using the "t" test according to the method of Snedecor and Cochran (1967).

RESULTS

Bacteriolological investigations: Bacteriological examination of 108 organ specimens (18 intestines, 18 livers, 18 lungs, 18 spleens, 18 kidneys and 18 hearts) collected from 18 necropsied local breed sheep, that had history of severe diarrhoea and fever, revealed the isolation of *E. coli* in 13/18 (72.2%) of the specimens, whereas in the faecal samples from diarrhoeic and contact apparently healthy sheep the incidence of *E. coli* was 42/59 (71.2%) and 17/35 (48.6%) respectively (Table 1). The 72 isolates were affiliated to 9 serogroups, O76 (13), O16 (10), O5 (10), O28 (8), O117 (7), O145 (5), O91 (3), O6 (3), O157 (3) and untypable isolates (10). Examining the adhering capacity of these isolated on two cell models, sheep enterocytes and HEp-2 cells as well as its capacity to produce verotoxines, revealed that all serogroups attached better on enterocytes than on HEp-2 cells, as 53/72 (73.6%) of the isolates adhered with an average of 63.5±6.2 *E. coli*/

enterocyte. On HEp-2 cells the adhesion of the isolates was significantly lower ($P < 0.05$) as 45/72 (62.5%) of the isolates were adherent with an average of 53.9±6.7 *E. coli*/ HEp-2 cell. Testing all the isolates for verotoxins production by the vero cell assay, revealed that 41/72 (56%) of the isolates were verotoxin producing. (Table2). Testing all the isolates for verotoxins production by the vero cell assay, revealed that 41/72 (56%) of the isolates were verotoxin producing. (Table2). The OMPs extract of the O76, O16 and O5 revealed similar protein band profile with more dense peptide bands between the range of 35kDa to 42 kDa. Lighter peptide bands were demonstrated in the SDS-PAGE gels at the higher and lower ends. In the Immune blotting examination, strong immunogenic protein bands 35-37kDa and 40-42kDa were demonstrated against sera from infected sheep as well as from sera from rabbits immunized with a pool from the extracted OMPs (Fig. 1). Sera collected from apparently healthy sheep reacted weakly against these immunogenic protein bands compared to the infected sheep as well as the immunized rabbits. Using sera from immunized rabbits as adhesion inhibitors in the previously mentioned cell models revealed significant inhibition ($P<0.01$) of the adhesion pattern that averaged 63.7% reduction in the adhesion capacity in case of enterocyte cell model (23.0±5.7 *E. coli*/cell), whereas the reduction in adhesion averaged 52.3% in case of HEp-2 cell model (20.2±3.4 *E. coli*/cell).

Post-mortem findings: Congestion and haemorrhages were seen on the surface of small and large intestines, which was associated with multiple erosions. The hepatic and kidney tissues were also congested and spleen was enlarged and haemorrhagic. Lungs showed multiple patchy congested areas on the pulmonary lobes.

Histopathological findings: In ilium and duodenal parts of small intestines there was coagulative necrosis of the upper part of mucosal villi associated with inflammatory cells infiltration in the submucosal layer (Fig. 2). Also there was diffuse desquamation and erosion in the epithelial cells lining the mucosal layer of the villi. These features were also associated with massive number of mononuclear leucocytic inflammatory cells infiltrating the underlining lamina propria (Fig. 3). It was also observed, appearance of aggregation of epithelioid and lymphoid cells in focal manner, also in the lamina propria (Fig. 4). In lung, bronchioles and air alveoli were impacted with inflammatory cells, also focal stratification of the epithelial cells lining the bronchioles associated with focal desquamation and ulcerations in the others. Interstitial stroma was thickened and infiltrated with neutrophils and macrophages (Fig. 5). In the liver, tissue sections revealed that the portal area of the liver was infiltrated by macrophages and lymphocytes. Also diffuse proliferation of kupffer cells was monitored (Fig. 6). Kidney tissue sections showed loss of cell details with coagulative necrosis of the epithelium lining

the renal tubules, which also appeared with deeply eosinophilic cytoplasm and pyknotic nuclei and in some cases with complete absence of the nuclei (Fig. 7). In the heart, myocardial bundles were infiltrated with neutrophils, macrophages, and few lymphocytes in focal manner (Fig. 8). Spleen sections showed depletion of lymphocytes in the white pulps (Fig. 9).

(Table 1) Pattern of *E. coli* serogroups isolation from different specimens.

E. coli Serogroup	Isolation from organs of (18) necropsied sheep	Faeces from (59) diarrhoeic sheep	contact apparently healthy (35) sheep	Total (%)
O76	3/18* (16.7%)	7/59* (11.9%)	3/35* (8.6%)	13/112 (11.6%)
O16	2/18 (11.1%)	6/59 (10.2%)	2/35 (5.7%)	10/112 (8.9%)
O5	2/18 (11.1%)	5/59 (8.5%)	3/35 (8.6%)	10/112 (8.9%)
O28	2/18 (11.1%)	4/59 (6.8%)	2/35 (5.7%)	8/112 (7.1%)
O117	1/18 (5.6%)	5/59 (8.5%)	1/35 (2.9%)	7/112 (6.3%)
O145	1/18 (5.6%)	4/59 (6.8%)	-	5/112 (4.5%)
O91	-	3/59 (5.1%)	-	3/112 (2.7%)
O6	-	-	3/35 (8.6%)	3/112 (2.7%)
O157	-	3/59 (5.1%)	-	3/112 (2.7%)
untypable	2/18 (11.1%)	5/59 (8.5%)	3/35 (8.6%)	10/112 (8.9%)
Total	13/18 (72.2%)	42/59 (71.2%)	17/35 (48.6%)	72/112 (64.3%)

* number of positive/ total number.

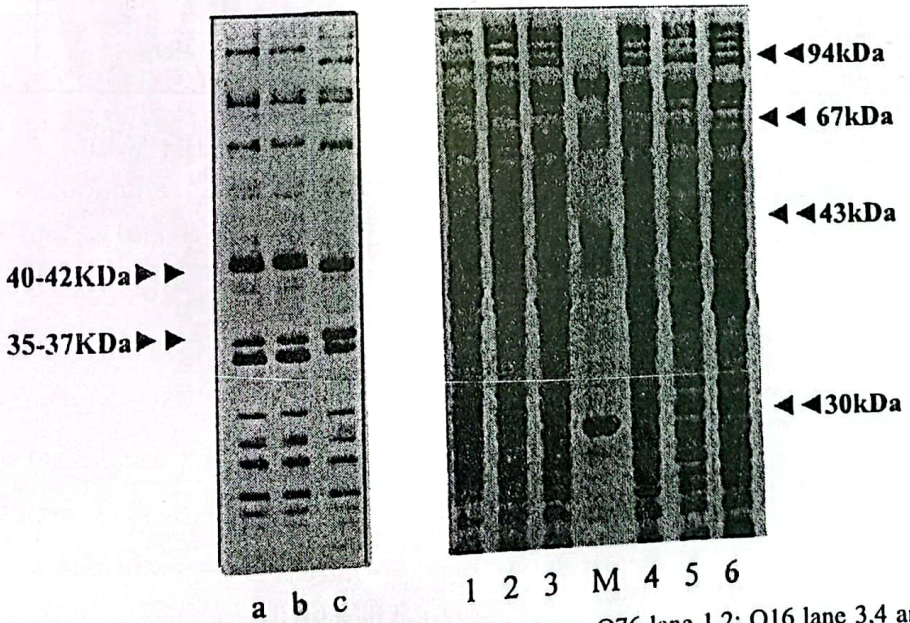

Fig. (1): SDS-PAGE of the OMPs of 6 *E. coli* isolates, O76 lane 1,2; O16 lane 3,4 and O5 lane 5,6. M is molecular weight protein marker (Pharmacia). Lane a, b and c are the immunoblotting against serum from immunized rabbits.

(Table 2): Pattern of some virulence factors of isolated *E. coli.*

E. coli Serogroup (number)	Ahhesion on enterocytes		Adhesion to HEp-2 cells		Verotoxins Production
	Without OMPs antibodies	Inhibition with OMPs antibodies	Without OMPs antibodies	Inhibition with OMPs antibodies	
O76 (13)	9/13[a] (60.9±9.6)[b]	(25.1±8.7)[b] 58.8%[c]	9/13 (58.1±8.9)	(24.7±6.5) 57.5%	7/13 (53.8%)
O16 (10)	7/10 (65.5±11.5)	(23.6±6.7) 64.0%	5/10 (51.8±5.2)	(18.8±3.4) 63.7%	6/10(60%)
O5 (10)	8/10 (59.6±10.4)	(24.9±8.2) 58.2%	6/10 (52.0±8.5)	(17.7±3.1) 34.3%	6/10(60%)
O28 (8)	5/8 (71.0±4.5)	(22.6±6.8) 68.2%	5/8 (55.3±8.2)	(20.2±4.9) 63.5%	4/8 (50%)
O117 (7)	7/7 (64.6±9.1)	(24.1±7.0) 63.1%	6/7 (49.8±7.1)	(20.3±4.8) 59.2%	3/7(42.9%)
O145 (5)	5/5 (65.4±4.5)	(20.6±4.4) 68.5%	4/5 (58.5±6.8)	(17.8±1.9) 69.6%	5/5(100%)
O91 (3)	2/3 (67.5±3.5)	(25.5±2.5) 62.2%	2/3 (57.0±4.0)	(19.5±1.5) 65.8%	1/3(33.3%)
O6 (3)	2/3 (64.5±0.5)	(22.5±3.5) 65.1%	2/3 (55.5±4.5)	(18.0±2.0) 67.6%	3/3(100%)
O157 (3)	2/3 (62.5±1.5)	(19.5±2.5) 68.8%	2/3 (54.5±6.5)	(17.5±1.5) 67.9%	3/3 (100%)
untypable (10)	6/9 (53.3±6.4)	(21.3±6.6) 60.0%	4/9 (46.9±7.3)	(27.7±4.8) 40.9%	3/10(33.3%)
Total (72)	53/72 (73.6%) (63.5±6.2)	(23.0±5.7) 63.7%	45/72 (62.5%) (53.9±6.7)	(20.2±3.4) 59%	41/72 (56.9%)

a) number of adhering isolates/ total number b) average number of bacteria/cell ± standard deviation

c) Inhibition %

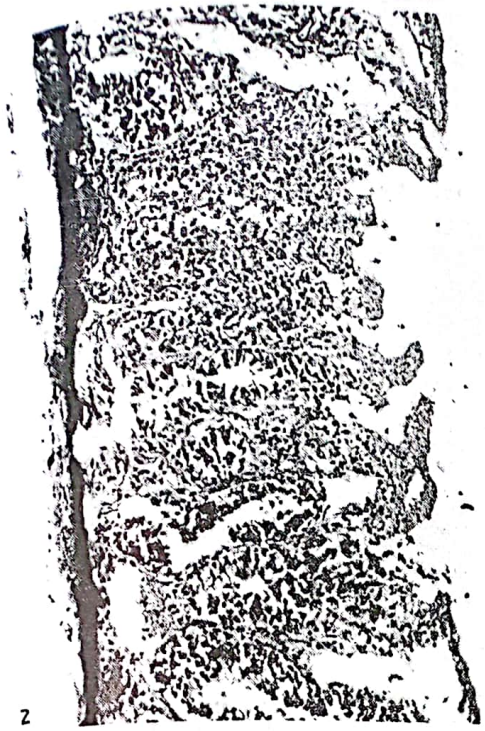

Fig.2. Small intestine (Duodenum) of sheep showing necrosis in the mucosal layer associated with inflammatory cells infiltration in submucosal layer (H&E x40).

Fig.3. Duodenum infected with *E. coli* showing desquamation and erosion of the mucosal lining epithelium of the villi with massive number of mononuclear leucocytic inflammatory cells infiltration in the lamina propria (H&E x40).

Fig.4. Small intestine (ilium) of sheep infected with *E. coli* showing aggregated lymphoid and epitheliod cells in the lamina propria (H&E 40x).

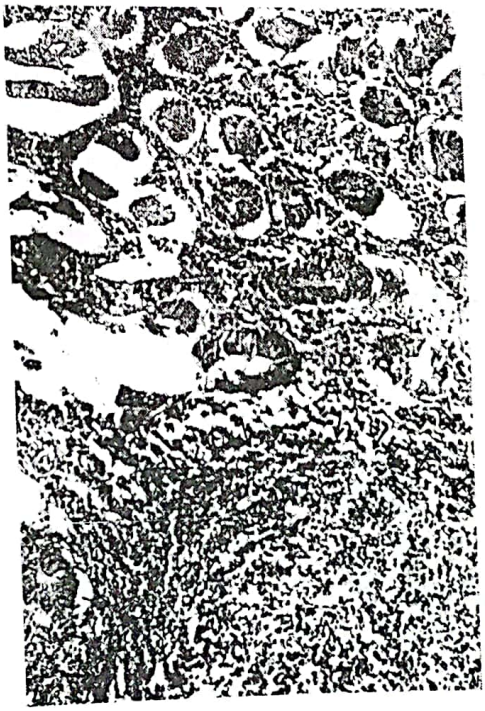

Fig. 5. Lung of sheep showing thickening in the interstitial stroma with inflammatory cells and fibrosis (H&E x40).

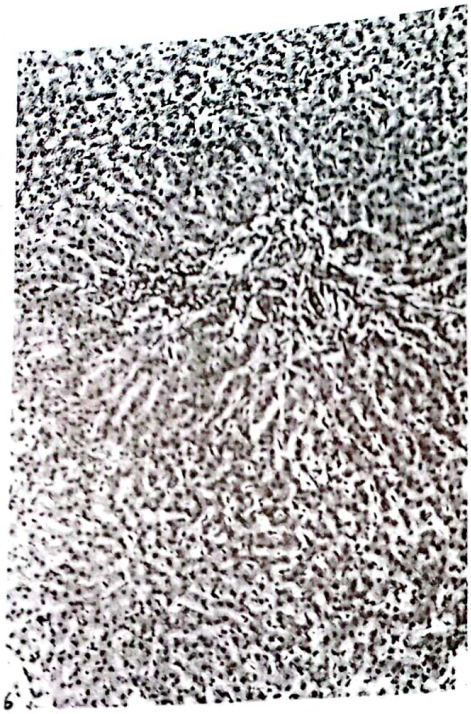

Fig. 6. Liver showing Kupffer cells proliferation (H&E x40).

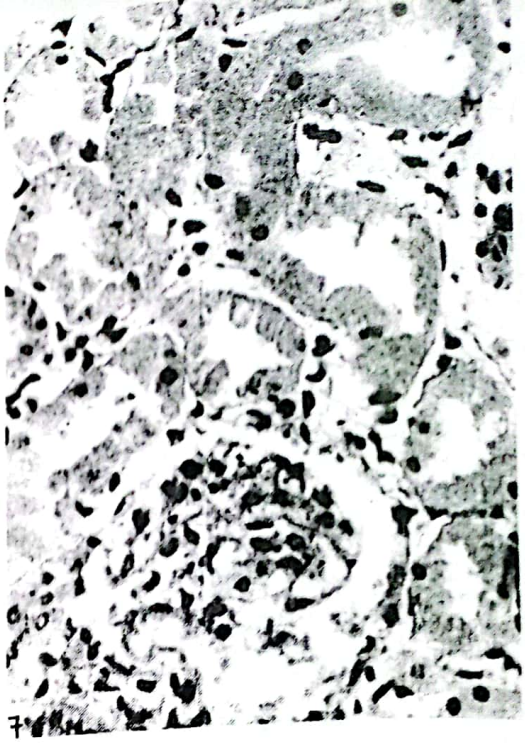

Fig.7. Kidney showing coagulative necrosis of the epithelial cells lining the renal tubules (high magnification, H&E x160)

Fig.8. Heart of sheep showing leucocytic inflammatory cell infiltration inbetween the myocardial muscle bundles (H&E x160).

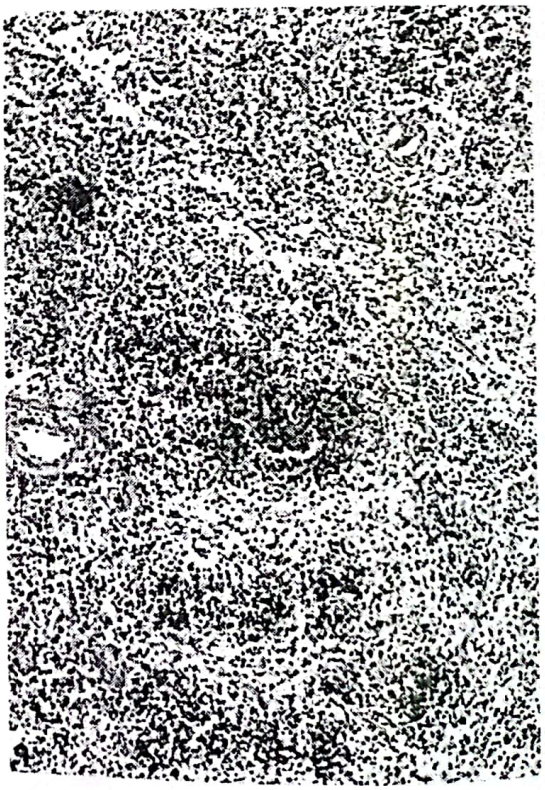

Fig. 9. Spleen showing depletion in lymphocytes of the white pulps (H&E x40).

DISCUSSION

Infection with *E. coli* in sheep is not only causing losses in sheep flocks but also is a serious and fatal public health hazard that can cause severe illnesses in humans through consumption of contaminated food products (Zweifel and Stephan 2003). Infected sheep had history of severe diarrhoea, fever that reached 41°C and anorexia. In this study the bacteriolological examination of 108 organ specimens (18 intestine, 18 liver, 18 lung, 18 spleen, 18 kidney and 18 heart) collected from 18 necropsied sheep revealed the isolation of *E. coli* in 13/18 (72.2%) of the necropsied animals. Also faecal samples were collected from diarrhoeic sheep as well as from contact apparently healthy ones for the isolation and identification of *E. coli* which was detected in 42/59 (71.2%) and 17/35 (48.6%) respectively (Table 1). The high incidence of *E. coli* in the specimens belonged to 9 serogroups, O76 (13), O16 (10), O5 (10), O28 (8), O117 (7), O145 (5), O91 (3), O6 (3), O157 (3) and untypable isolates (10). Similar isolation patterns in sheep were reported by Bettelheim et al., (2000) Rey et al., (2003) and Wani et al., (2003) who diagnosed similar *E. coli* infections in sheep flocks with a wide diversity of serogroups that induced the infection. Investigating the adhesion capacities of the isolates on two cell models, namely sheep enterocytes and HEp-2 cells, revealed that all serogroups attached better to enterocytes than to HEp-2 cells, as 53/72 (73.6%) of the isolates adhered with an average of 63.5±6.2 *E. coli*/ enterocyte. While on HEp-2 cells the adhesion was significantly lower ($P<0.05$) as 45/72 (62.5%) of the isolates were adherent with an average of 53.9±6.7 *E. coli*/ cell. These findings could be explained as sheep enterocytes might have more specific receptors for the invading *E. coli* than the HEp-2 cells. These findings were supported by Gyles (1994) and Ruttler et al., (2002) who also recorded higher attachment pattern of different *E. coli* serogroups to sheep enterocytes than any other cell model. Testing all the isolates for verotoxins production by detection of cytopathic effect (CPE) on the vero cells, revealed that 41/72 (56%) of the isolates were verotoxin producer (Table2) which is very hazardous factor that was encoded by phages that can integrate its genes as stx1 and stx2 into the bacterial chromosomes. Verotoxin production was not stable virulence chriteria as the encoding genes could be lost over time or could be switched off. Similar verotoxin production pattern was also reported by different research groups (Zhu et al., 1994; Munoz et al.,1996; Bettelheim et al., 2000, Seleim and Mohamed 2003; Vettorato et al., 2003).

The OMPs extract of the O76, O16 and O5 which were the dominant serogroups revealed similar protein band profile with more dense peptide bands between the range of 35kDa to 42 kDa. Lighter peptide bands were demonstrated in the SDS-PAGE gels at the higher and lower ends. This was also demonstrated by Gyles (1994)

Hellman et al., (2000) who confirmed the similarity of different *E. coli* serogroups in their OMPs protein pattern which conferes many virulence characters as serum resistence, colicins production, phage receptors, porins or iron chelating proteins. Zhao et al., (1996) revealed similar OMPs from O28 and O157 that were of virulence characteristics. Also in the Immune blotting examination,this study demonstrated strong immunogenic protein bands 35-37kDa and 40-42kDa against sera from infected sheep as well as from sera from rabbits immunized with a pool from the extracted OMPs (Fig. 1). Sera collected from apparently healthy sheep reacted weakly against these immunogenic protein bands compared to the infected sheep as well as the immunized rabbits. However, these findings confirmed the immunogenicity of these bands and the suitability of application of these OMPs preparations as protective vaccine that can elicit protective immune response. Using sera from immunized rabbits as adhesion inhibitors in the previously mentioned cell models revealed significant inhibition ($P<0.01$) of the adhesion pattern that averaged 63.7% reduction in the adhesion capacity in case of enterocyte cell model (23.0±5.7 *E. coli*/ cell), whereas the reduction in adhesion averaged 52.3% in case of HEp-2 cell model (20.2±3.4 *E. coli*/cell). These finding confirm the suitability of OMPs as potential vaccine against *E. coli* infection which was also supported by Kawahara et al.,(1994) Pugh and Wells (1985) Deproy et al., (1995) Hellman et al., (2000).

The histopathological finding of the necropsied sheep demonstrated small intestine (duodenum), there was coagulative necrosis associated with inflammatory cells infiltration in the submucosal layer (Fig. 2). Also diffuse desquamation and erosion in the epithelial cells lining with destruction of the duodenal villi. These features were also associated with massive number of mononuclear leucocytic inflammatory cells infiltrating the underlining lamina propria (Fig. 3) This could be due to the different *E. coli* virulence characteristics as toxin production as reported by Cookson et al., (2002). It was also observed in the ilium, appearance of epithelioid and lymphoid cells in focal manner, also in the lamina propria (Fig. 4) These structure was also reported by Glasser et al., (2001) and Thiede and Krone (2001). In lungs there was focal stratification of the epithelial cells lining the bronchioles associated with focal desquamation and ulcerations in the others. Interstitial stroma was thickened and infiltrated with neutrophils and macrophages (Fig. 5) which could be attributed to the effect of general systematic infection. Liver, tissue sections revealed diffuse proliferation of Kupffer cells (Fig. 6) which was similar to findings reported by Cookson et al., (2002) Sokkar et al., (2003). Kidney tissue showed loss of cell details with coagulative necrosis of the the renal tubules, which had deeply eosinophilic cytoplasm and pyknotic nuclei. In some cases complete nuclei absence (Fig. 7). In the heart, myocardial bundles were infiltrated with neutrophils, macrophages,

and few lymphocytes in focal manner (Fig. 8). Spleen sections showed depletion of lymphocytes in the white pulps (Fig. 9) which was also supported by Martin and Aitken, (2000). It was concluded that attaching and enterotoxigenic *E. coli* could be the cause of mortality and diarrhoea in the under studied sheep flocks, that was evidently supported by the diverse isolation of different virulent E. coli serogroups which demonstrated crucial virulence factors. Also the potential effect of using OMPs as candidate vaccine for controlling the infection with *E. coli* in sheep was highlighted.

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