RESULTS

The study included 42 patients clinically suspected of having enteric fever . Out of the 42 clinically suspected cases, 10 (23.8%) patients had positive blood culture for salmonella typhi. The rest of the clinically suspected cases, 32, had negative blood culture with a percentage of 76.2%.

Table (2) : shows the results of the blood culture of clinically suspected enteric cases.

The 10 strains of S. typhi were tested for presence of Vi antigen and they were found positive. Preparation of Vi and LPS antigens from the isolated strains was done.

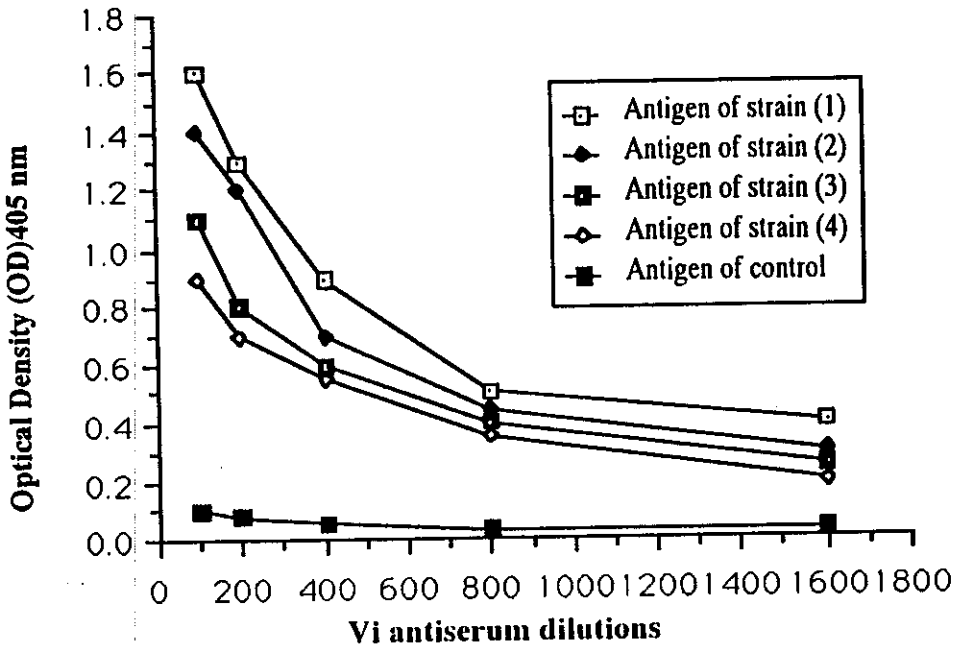
Fig. (2) shows the results of ELISA test for detection of S. typhi Vi antigen and the relation between serum dilution of specific Vi antisera for S. typhi and optical density (OD) to the different preparations of antigens. 4 strains show higher values and these strains were used for future study.

Protein concentration of the different 4 preparation of Vi antigens of S. typhi was as follows. Protein content of number 1 = 150 μg/mL, number 2 = 85 μg/mL; number 3 = 77.6 μg/mL and number 4 = 26 μg/mL.

Table (2) : The results of blood culture for clinically suspected enteric fever cases.

Positive blood culture		Negative blood culture		Total number of cases
No.	%	No.	%	
10	23.8	32	76.2	42

Fig. (2) The results of ELISA test for detection of S. typhi Vi antigens.



- **Demonstration of protein bands by silver stain for Vi antigens of 4 preparation of S. typhi** :

The molecular weight of the reacted bands were determined from the standard curve of blue stained protein markers which have the molecular weight 106, 80, 49.5, 32.5, 27.5 and 18.5 Kilodaltons (KDa) by the calculation of the mobility of the protein and of the marker dye; the retardation factor was calculated as (Weber K and Osborn, 1969).

Retardation Factor (RF)

$$= \frac{\text{Distance Travelled by protein}}{\text{Distance travelled by dye}} = \frac{S}{D}$$

The mobilites were plotted against the known molecular weights expressed on a semi-logarithmic scale.

The 4 strains showed almost the same pattern on the gel; number 3 and number 4 showed faint bands than number 1 and number 2. The molecular weights of the resulting bands 85, 41, 34, 26 and < 10 KDa as shown in Fig. (3).

Immunoblotting of Vi antigens of S.typhi on nitrocellulose sheet with specific Vi antiserum of S. typhi showed the reaction with the bands located at 85, 34, 26 and <10 KDa . The result of using antisera of shigella and antisera of brucella to see the degree of cross reactivity compared with immunoblotting of Vi antigen of S. typhi showed the following results:

- No bands appeared with Brucella abortus agglutinating serum and Brucella melitensis agglutinating serum.
- No bands with Shigella agglutinating serum { Dysenteriae polyvalent (3-10)} and with shigella agglutinating serum {Flexneri polyvalent (1-6, x &y)}.
- 2 bands located at 34, and < 10 KDa with shigella agglutinating serum {Sonnei phase 1&2}.
- One band located at less than 10 KDa with shigella agglutinating serum {Boydii polyvalent 1 (1-6); Bodyii polyvalent 2 (7-11) and Boydii polyvalent 3 (12-15)}.

These results suggests that there is a degree of cross reactivity between Vi antigens of S. typhi and shigella sonnei phase 1&2 and shigella boydii polyvalent 1,2,3 as shigella sonnei share with Vi antigens of S. typhi at this study in protein bands located at 34 and less than 10 KDa, and also between it and shigella boydii polyvalent 1,2,3 in protein band located at less than 10 thousand KDa.

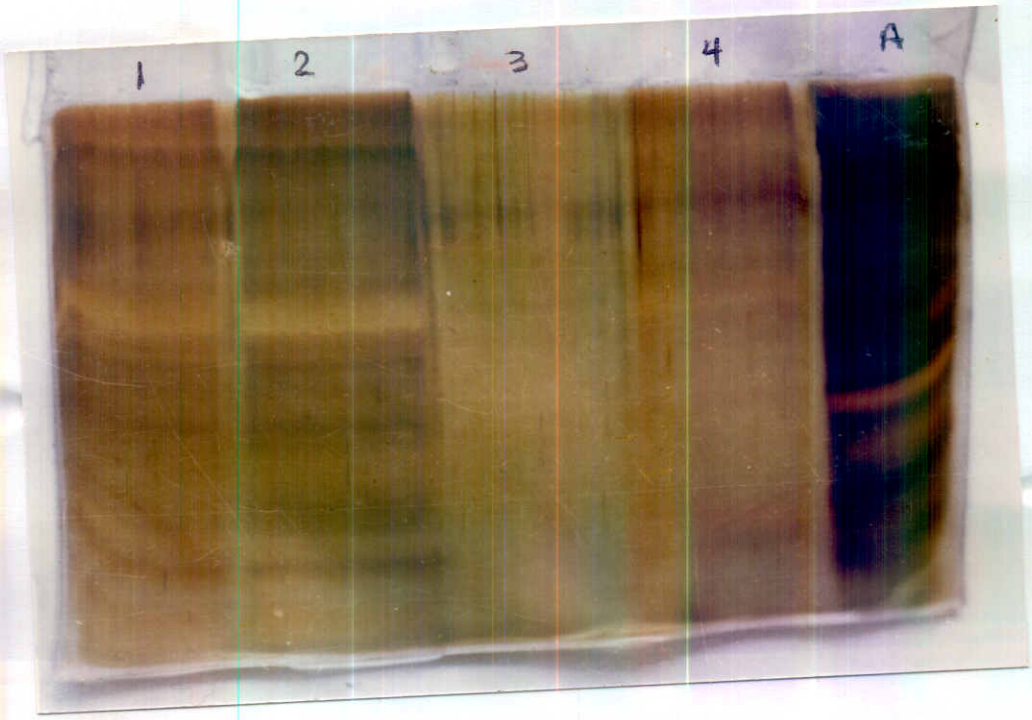
- **Demonstration of protein bands by silver stain for Crude LPS preparation of 4 strains of S. typhi**:

The mobilities of different proteins were plotted against the known molecular weight expressed on a logarthmic scale.

The molecular weights of the resulting bands,> 106, 80, 60, 48, 24 and 17 KDa. as shown in Fig. (4).

The 4 preparations showed almost the same bands on silver

Fig.(3) Silver stain for Vi antigens of S. typhi



Lane A = Standrad marker of known molecular weights.

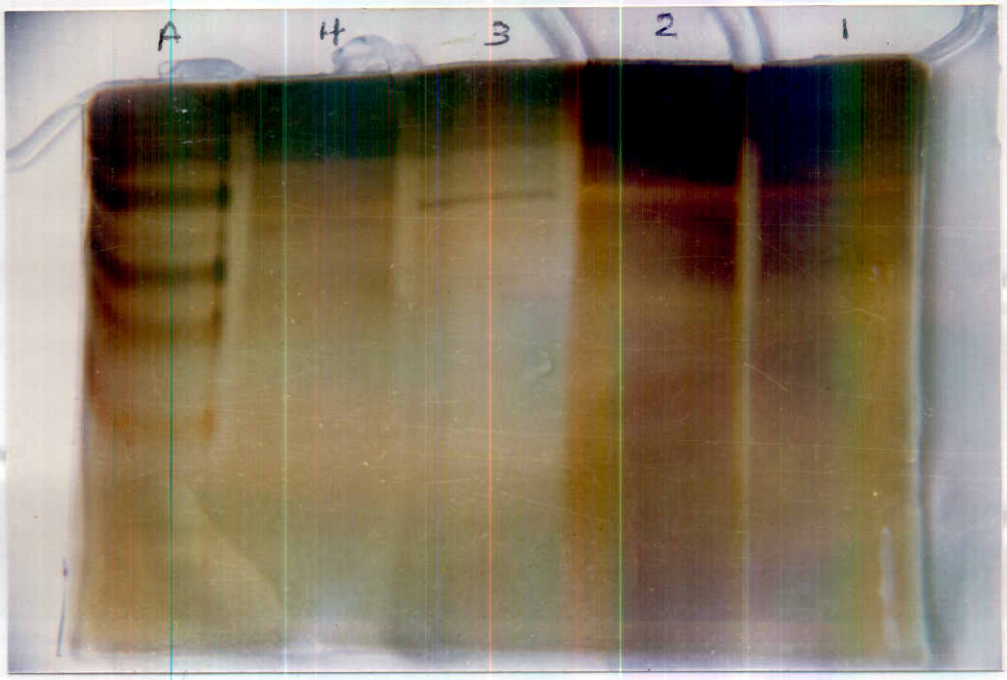
Lane 1 = Vi antigen no. 1 of S. typhi .

Lane 2 = Vi antigen no. 2 of S. typhi

Lane 3 = Vi antigen no. 3 of S. typhi

Lane 4 = Vi antigen no. 4 of S. typhi

Fig.(4) Silver stain for LPS antigens of S. typhi



Lane A = Standrad marker of known molecular weights.

Lane 1 = LPS antigen no. 1 of S. typhi .

Lane 2 = LPS antigen no. 2 of S. typhi

Lane 3 = LPS antigen no. 3 of S. typhi

Lane 4 = LPS antigen no. 4 of S. typhi

Table (3) : The results of plating of the four strains of S. typhi (Diluted 10^{-6}) and non invasine strain of E. Coli.

In put plating of strains	no. of colonies on Muller Hinton (CFU)	no of colonies on MacConkey (CFU)	Average (CFU)
No.1 of S. typhi	18	28	18
No.2 of S. typhi	25	25	25
No.3 of S. typhi	24	24	24
No.4 of S. typhi	28	26	27
Non invasive E. Coli	18	20	19

CFU = Colony Forming Unit.

Table (4) : The results of plating of the strains of S. typhi (Diluted to 10^{-2}) and non invasine strain of E. Coli.

Out put plating of strains	no. of colonies on Muller Hinton (CFU)	no of colonies on MacConkey (CFU)	Average (CFU)
No.1 of S. typhi	37	35	36
No.2 of S. typhi	58	62	60
No.3 of S. typhi	5	5	5
No.4 of S. typhi	8	6	7
Non invasive E. Coli	3	3	3

Table (5) The results of input isolated strains of S.typhi

Isolates	CFU / plate	Dilution	Titer / m.L / μL.	ul of diluted culture / well	In put CFU / well
No. 1 of S. typhi	18	10^{-7}	18×10^7/mL 18×10^4/μL	2	36×10^4
No. 2 of S. typhi	25	10^{-7}	25×10^7/mL 25×10^4/μL	2	50×10^4
No. 2 of S. typhi	24	10^{-7}	24×10^7/mL 24×10^4/μL	2	48×10^4
No. 2 of S. typhi	27	10^{-7}	27×10^7/mL 27×10^4/μL	2	54×10^4
Non invasive E. Coli	19	10^{-7}	19×10^7/mL 19×10^4/μL	2	38×10^4

The results of output isolated strains were estimated as in table (6).

Isolates	CFU / plate	dilution	Output CFU / well
No.1 of S. typhi	36	10^{-3}	36×10^3
No.2 of S. typhi	60	10^{-3}	60×10^3
No.3 of S. typhi	5	10^{-3}	5×10^3
No.4 of S. typhi	7	10^{-3}	7×10^3
Non invasive Strain E. Coli	19	10^{-1}	19 x 10

The invasion power was calculated by the following equation power of invasion :

$$= \frac{\text{No. of out put (CFU/well)}}{\text{No. of input (CFU/well)}}$$

No.1 of S. typhi had invasive power about 10%, No.2 of S. typhi 12%, No. 3 of S. typhi and No.4 showed a less invasive power 1.04% and 1.5% respectively, the non invasive E.Coli strain showed a non significant value of invasion about 0.05% and used as negative control.

Results of tissue culture cell cytotoxin assay by S. typhi strains :

The 4 strains of S. typhi were tested for their ability to produce toxins on Henle 407 human intestinal cell by observing the morphological changes that occur to monolayer cells with inverted microscope.

In strain no.1 the use of bacterial supernatant without dilution showed complete detachment of monolayer cells., the 1/10 dilution of this filterate showed partial detachment of cells. The same effect was seen on 1/50 dilution.

In strain no.2 showed complete detachment of monolayer cells on using undiluted supernatant. In the wells which were diluted 1/10, 1/50 there was partial detachment of cells.

In strain no.3 the well without dilution of supernatant bacterial filtrate showed complete detachment of monolayer cells while in well

with 1/10 and 1/50 dilutions were shown that the monolayer cells was intact in most of the well.

In strain no. 4 there was complete detachment of monolayer cells. In the wells which were diluted 1/10 and 1/50 were shown that the monolayer was intact of the most of the well.

In Muller Hinton control well, the monolayer cell was intact and no detachment was seen; cells looked healthy.

From these morophological differences between strain no.1 and strain no.2 of S. typhi and strain no.3 and no.4, may suggest the production of Toxins by strain no.1 and no.2 and these results may suggest the role of toxin production in pathogenesis of S. typhi.

DISCUSSION

The Lipopolysaccharides (LPS) of Salmonella species and other members of the family Enterobacteriaceae are complex molecules comprising three structurally and serological distinct regions i.e a lipid moiety, a core oligosaccharide, and the O-specific side chain. Although there are over 2,000 serologically distinct species, it is believed that in salmonella genus there is only one core structure which shows serological cross-reactivity with some members of the Enterobacteriaceae (Schmidt et al., 1970). In salmonella infections, the lipopolysaccharide (LPS) is of importance for both pathogenicity and virulence (Lindberg, A.A., 1980).

The Vi capsular polysaccharid is a component of several members of the family Enterobacteriaceae. The Vi capsular polysaccharide is both a virulence factor and a protective antigen of S. typhi (Daniels et al., 1989).

Vi antigens, so prepared by Baker et al, 1959 appeared to be electrophoretically homogeneous, had essentially constant analytic properties, had a fairly narrow equivalence zone and seemed to be serologically homogeneous. It may be noted that, with respect to nitrogen content, the various preparation of S. typhi antigens were similar, whether prepared from the same or different organisms.

In our study , the Vi antigens prepared from 4 strains of S. typhi showed almost the same bands on polyacrylamide gel.

It has been suggested that bacteria of Enterobacteriaceae may have the same R core structure as that found for salmonella. The core structure of shigella flexneri serotypes with a comparison with the corresponding core portion of salmonella showed that it is built up of the same sugar constituents but in a different way. Nevertheless the general make-up of the two structures strongly suggests a close evolutionary relationship between these two bacterial groups. (Schmidt et al., 1970).

In our study there is a degree of cross reactivity between LPS antigen of S. typhi and shigella flexneri as they are shared in two bands on nitrocellulose sheets after immunoblotting.

The results of the absorption tests indicated that the cross-reacting antigenic determinants of brucella, yersinia and group N salmonella strains were associated with the lipopolysaccharide containing agglutinogen complexes. The results of fluorescent antibody tests confirmed the distant antigenic relationship between the group N salmonellae and smooth Brucella species compared to the closer antigenic relationship between the latter and Y. enterocolitica IX. The O30 side chains of group N salmonella lipopolysaccharides have been shown to contain glucose residues in β1-3 and β1-4 linkage to N- acetylgalactosamine, it seems possible that the antigenic determinants involved is perhaps linked through galactosyl residues to the core of the lipopolysaccharide agglutinogen (Corbel M.J., 1975).

Hurvell et al, (1971) confirmed the antigenic relationship between Brucella abortus, Brucella melitensis and Brucella suis and Y. enterocolitica 09 in agglutination and complement fixation tests.

Serological cross-reactions have been domenstrated between Brucella species and E. coli 0:116 and 0:157; francisella tularensis; Salmonella serotypes of kauffman-white group N (0:30 antigen); Pseudomonas maltophilia, Vibrio cholerae and Yersinia enterocolitica serogroup 0:9 (Topley and Wilson's , 1983).

The subclass distribution for specific antibodies to salmonella serogroup B and D O Lipopolysaccharides in serum was Ig G_1 and Ig A_1 and Ig A_2. The corresponding pattern for antibodies to shigella flexneri O Lipopolysaccharides was Ig G_1, Ig G_2 and Ig A_1 (Persson et al., 1986).

Shigella sonnei is a predominant cause of bacillary dysentery in the United States and worldwide. The major protective shigella sonnei surface antigen, the form I LPS has been chemically defined to contain an unusual O-disaccharide repeat unit linked to core lipid A. There are no proven effective prophylactic measures to protect against this intestinal mucosal disease. In an attempt to construct a vaccine that would stimulate local intestinal immunity to Sh. sonnei the genes determining the shigella from I antigen have been transferred to an attenuated mutant strain of salmonella typhi, Ty21a (Seid et al., 1984).

The LPS complex was identified as one of the immunodominant components and has been widely used for serological tests. Yet the considerable antigenic overlap between brucella species and other

Gram-negative bacteria complicates the development of antibody reagents useful for identifying Brucella organisms at the species level. The biochemical basis of serological cross-reactions between B. abortus and Y. enterocolitica serotype 0:9 was shown to be due to a common polysaccharide moiety localised on the O antigen. Isolation of LPS from Y. enterocolitica 0:9 and from B. abortus led to the chemical identification of the common polysaccharide units (Wilke et al., 1987).

The LPS outer core structure of smooth S. typhi and shigella boydii type 3 and shigella flexneri showed sharing structural similarity (Tsang et al., 1987).

In our study there is a cross reactivity between shigella bodydii type 1,2,3 and LPS of S. typhi as sharing in 2 bands of immunoblotting on the nitro cellulose sheet.

Dodds et al. (1987) stated that two chemically different O-polysaccharides, a low molecular mass form of LPS and core LPS produced by chemostat-group E. Coli 0157, were analysed by SDS-PAGE, silver staining and immunoblotting. The reactivities of the different O-polysaccharides with antisera prepared against E. Coli 0157 group in batch culture, salmonella 030 or Brucella abortus were very similar, showing that the O-polysaccharides share at least some antigenic determinants. The reactions of the low molecular mass LPS with the antisera indicated it was semi-rough LPS having one repeat unit of the O-polysaccharide attached to core LPS.

Protection against enteric pathogens like shigella may be

determined by the production of secretory immunoglobulins at the level of the intestinal mucosa, where they may prevent adherence of the bacterium or interfer with bacterial toxin. The S. typhi-Shigella sonnei bivalent vaccine strain has been shown to express O antigens from both S. typhi and sh. sonnei. Vacciness had serum and local intestinal immune responses to Sh. Sonnei lipopolysaccharide and the presence of specific serum IgA or IgG antibody before challenge with pathogenic Sh. sonnei was correlated with protection from illness (Black et al., 1987).

Qadri et al. (1988) stated that four monoclonal antibodies (MAbs) showed a considerable degree of cross-reactivity recognizing S. typhi, S. paratyphi A, S. paratyphi B and E. Coli and Shigella sonnei.

In our study there is sharing between Vi and crude LPS of S. typhi and Shigella sonnei; two bands for each antigen as shown by immunoblotting on nitrocelluose sheets.

The lipopolysacchaide of S. urbana and S. godesberg which belong in group N (0:30) of the kanffmann-white system, were shown by SDS-PAGE electrophoresis. The serological cross-reactivity of S. urbana and S. godesberg with Brucella abortus and Y. enterocolitica (0:9) can now be related to the presence of mannopyranosyl residues in their respective lipopolysaccharide O-chains (Perry et al., 1986).

Electrophorteic separation of Brucella abortus proteins using SDS-PAGE analysis resulted in separation of multiple bands. Bands corresponding to proteins (94, 43, 30 and 14 KDa) were demonstrated

(Alder et al., 1988).

The smooth LPS were analysed by SDS-PAGE and silver staining. Immunoblott were developed by using either monoclonal antibodies specific for brucella A or M antigens or polyclonal polyspecific or monospecific sera from rabbits, cattle and goats. The most represented subunits of S-LPS ranged form 30 to 70 KDa relative to marker proteins (Baskuji et al., 1990).

In our study there is no cross reactivity between Vi and crude LPS of S. typhi (group D) and Brucella abortus or Brucella melitensis as there is no sharing bands appear between them by immunoblotting.

It had been found that there is a greater degree of homogenicity between shigella species and E. Coli than between either of these species and salmonella species. (Scultz C., 1989).

The crude preparation of S. typhi antigens showed the presence of several protein components with molecular weight that ranged between 70 and 13 KDa (Ekpo et al., 1990).

The SDS-PAGE pattern of S. typhi strains showed protein bands that are located at 17, 28 34 and 36 to 41 KDa proteins (Ortiz et al., 1989).

The analysis of Vi and LPS of S. typhi by SDS-PAGE and silver staining in the present study showed almost most of bands in other work studies by Ortiz et al (1989) and Ekpo et al (1990).

The Salmonella typhi-shigella sonnei hybrid vaccine was constructed to express the Sh. sonnei form I antigens, which may play

an important role in producing protective immunity. In fact, it was previously shown that this bivalent vaccine strain stimulated an intestinal immunoglobulin A respones to the Sh. sonnei form I antigen in rabbits and produced both serum and intestinal antibody responses to form I antigen in human vaccines (Hartman et al., 1991).

The serological cross-reactivity of E. Coli 0:157 with Brucella abortus, Y-enterocolitica (serotype 0:9), group N Salmonella, and some other E. Coli species can be related immunochemically to the presence of 1,2-glycosylated N-acylated 4-amino-4,6 dideoxy-alpha-D-mannopyranosyl residues in the O-chains of their respective Lipopolysaccharides (Perry et al., 1986).

A bivalent vaccine consisting of Salmonella typhi strain Ty21a containing the 120 MDa plasmid of shigella sonnei and expressing both S. typhi and Sh. Sonnei lipopolysaccharides on its surface was previously shown to protect significantly against Sh. sonnei disease in experimental challenge studies (Herrington et al., 1880).

The ability of bivalent salmonella typhi-shigella sonnei vaccine to stimulate on intestinal immunoglobulin A response in humans was evaluated by detecting gut-derived, trafficking antibody secreting cells (ASC) in peripheral blood (Van-de-verg et al., 1990).

Salmonella typhi is by for the major cause of enteric fever. Attempts to develop safe, practical and effective vaccines against these agents were under way. Examples of vaccine candidate include live oral vaccines against S. typhi, V. Cholerae, shigella, and rotavirus, and inactivated, submit vaccines given parentally or orally against S.

typhi, V. cholerae or Enteropathogenic E. Coli (Levine and Edelman, 1990).

The electrophoretic analysis of lipid A associated protein (LAP), obtained from shigella sonnei, in polyacrylamide gel in the presence of sodium dodecyl sulfate and urea has revealed the heterogeneity of the preparation, it has found to contain three main components with molecular weights of 43, 38 and 18 KD and minor components with molecular weights of 49, 45, 34, 30, 29, 27, 25, 21 and 14 KDa. As shown by the method of enzyme immunoassay, the antigenic affinity of LAP of different origin corresponds to the degree of taxonomic propinquity of microorganisms, the maximal degree of cross reactions is observed between LAP obtained from shigella sonnei, shigella flexneri,and E. Coli, while their affinity to salmonella typhi is considerably less, remote microbial species (Bacterium bifidum and Sarcina marcescens) give practically no cross reactions (Nartikova et al., 1991).

In this study, the immunoblotting with shigella species antisera including Sh. sonnei, Sh. Flexneri, Sh. bodydii 1,2,3 and Sh. dyesentriae against crude Vi and LPS antigens of S. typhi, showed that 2 bands sharing with Sh. sonnei, one band with Sh. boydii 1,2,3 while no bands with Sh. dyesentriae and Sh. flexneri and these with Vi antigen of S. typhi.

With LPS antigen of S. typhi there were also 2 bands sharing with Sh. sonnei, Sh. Flexneri and shigella bodydii. Sh. sonnei and Sh. Flexneri and to less extent Sh. bodydii showed a degree of cross reactivity with S. typhi antigens.

Jesudason et al.(1991) stated that cathodal moving protein components were identified in agarose gel of S. typhi. Rabbit antiserum was raised against these cation's proteins, it had both agglutinating and precipitating activity. A total of 80 Salmonella strains belonging to serogroups A, B, C_1 , C_2, D, E_1 and E_2 including 26 S. typhi and 10 S. paratyphi A were tested against this antiserum in a slide agglutination test; all strains were agglutinated. Among 49 other bacterial strains tested, the antiserum agglutinated all 16 strains of shigella Flexneri, 2 of 5 shigella sonnei, 5 of 34 E. Coli and 1 of 8 citrobacter species. These results show that there is antigenic sharing of the non flagellar proteins of S. typhi with many other shigella and E. Coli .

In the course of developing a live vaccine, there are three murine monoclonal antibodies (MAb) specific for shigella sonnei. The specificities of these MAb were determined by ELISA and immunoblot analysis with whole cell or purified lipopolysaccharide, as antigens. Two of them are specific for the shigella serotype D O-polysaccharide determinant, whereas one specifically binds to the core hexose region of LPS. With these MAb, it was possible to analyze clinical isolates and a hybrid Salmonella of the corresponding LPS moieties. In addition to their use in the screening of candidate vaccine strains; the new MAb provide a powerful tool for epidemiological and phylogenetic studies of natural enterobacterial populations .Minor differences in protein patterns were found among

S. typhi strains suggesting that envelope protein profiles may be of useful as adjuvant epidemiologic marker in outbreak investigations. However, it was not possible to correlate specific protein bands or profiles with severity of illness or the geographic source of the isolate (Franco et al., 1992).

Pathogenicity of Salmonella depend first upon their ability to penetrate the epithelium of the gastrointestinal tract and the to persist and multiply in the macrophages of the reticuloendothelial system. The most likely anatomical site of penentration in small intestine (Takenchi and Sprinz, 1967).

It is questionable whether the lipopolysaccharide is an important toxic agents in enteric fever or whether the antiphagocytic activity of the Vi antigen of significance in natural typhoid infection. Living salmonella may cause the acumulation of fluid in ligated loops of rabbit intestine, this occurs only when Salmonella invade the gut wall and cause inflammation, but not all invasive strains cause fluid accumulation. There is enterotoxin in sterile filtrates of Salmonella cultures that cause diarrhoea in suckling mice and fluid accumulation in rabbit ileal loops (Topely and Wilson, 1983).

Brucella toxins posses many structural and biological properties in common with endotoxin of the enterobacteriaceae. However, some quantitative differences in their biological potencies were observed. Fraction 5 from Brucella abortus and Brucella melitensis were endotoxins which possess many immunological in common with endotoxin extracted from the Enterobacteriaceae (Leong et al., 1970).

Brucella entering the host via skin abrasion or mucous membrane surfaces are phagocytized and transported to regional lymph nodes. They multiply within the phagocytes which lyse, leading to bacteraemia. Interacellular growth of the organisms in macrophages enables the pathogens to establish long-term infection despite the presence of antibody (Munford et al., 1979).

Brucella organisms produce no exotoxins but as in all gram-negative bacteria, the lipopolysaccharide (LPS) in their cell wall is an endotoxin. Brucella endotoxins have the some chemical and biologic properties as the endotoxins of enteric bacilli. A prominent feature of Brucella infection is intracellular parasitism of the macrophages and histiocytes of the reticuloendothelial system. Focal aggregates of the parasitized cells produce granulomas of the liver, spleen, and bone marrow (Ibraham, 1983).

Brucella resembles the tubercle bacillus and Listeria monocytogenes in growing intracellulary. The ability of virulent strains of Brucella abortus to resist the destructive action of phagocytes, and even to multiply within them, is ascribed to the presence of a substance in the cell wall of the bacterium that can be neutralized by a specific antiserum (Topley and Wilson, 1983).

Dysentry bacilli possess the intrinsic capacity to invade the mucous membrane of the gut. evidence is provided by the fact that this property can be transferred experimentally to non-invasive E. Coli. Surprisingly few bacterial cells of a virulent strain suffice to produce clinical illness in human volunteers. Several experimental models

have been used for the study of pathogenic activities of shigellae, such as oral infection of monkeys and starved guinea pigs, infection of the eye resulting in Keratoconjunctivities of the guinea pig, and infection of tissue culture cells. The particular nature of the invasive factor, however, has not been elucidated. It has been known for many years that the classical shiga bacillus produces an exotoxin that is lethal to the mouse and is considered to be a neurotoxin. Products of shigellae with cytotoxicity for Hela cells and enterotoxicity in the rabbit ileal loop test have been identified. Their role in human shigellosis remains moot (Felix M. and Thomas D, 1982).

The toxicity of the dysentery bacilli, like that of other enterobacteria, is associated in the main with the cell-wall lipopolysaccharide. In addition, shiga bacillus forms a toxic product which has been described as exotoxin or neurotoxin. The cytotoxic and enterotoxic activity of this agents may thus have role in the pathogenesis of bacillary dysentery (Topely and Wilson, 1983).

Two strains of shigella flexneri, one strain of shigella sonnei produced a cell-free cytotoxin which was neutralizyed by the neurotoxin of shigella dysentriae type I. The antibody activity was associated only with the Ig M fraction in Sh flexneri, Sh. sonnei and Sh dysentriae infections. The pathogenesis of shigellosis is related to the invasive properties of the organism (Kensch and Jacewicz, 1977).

It is not clear what role, if any shigella toxin plays in pathogenic changes, non are the pathogenic mechanisms of shigella-associated complications well delineated (Koster et al., 1984).

Shigella sonnei and shigella flexneri currently account for most cases of shigella-associated seizures although these species do not usually produce shiga toxin as demonstrated by toxin neutralization and DNA hybridzation studies (Neill et al., 1988).

Closely related shiga like toxins I and II are produced by certain E. Coli strains, they are functionally and structurally similar to shiga toxin, with the same biologic activities (O'brien and Holmes, 1987).

The neurologic symptoms in human shigellosis have often been attributed to shiga toxin, although its exact role has not yet been determined, by use of a thymidine labeled Hela cell assay, cytotoxic acitivity was demonstrated in stool but not cerebrospinal fluid or serum from five patients with shigellosis presenting with seizures or encephalopathy. Thus shiga toxin production is not essential for the development of neurologic manifestations of shigellosis, other toxin products may play a role (Ashkenazi et al., 1990).

Pathogenesis of Salmonella gastroenteritis is attributed mainly to production of E. coli. heat labile toxin (LT) like enterotoxin. Invasiveness, though important is thought to play a role secondary to the enterotoxin (Sharma et al., 1987).

Limited data are available concerning cytotoxin production by salmonella strains. Cytotoxin production has been demonstrated by O'Brien et al, (1982) in one S. typhimurium strain, by ketyi et al, (1979) in three S. enteritidis strains. Further studies have shown that Salmonella cytotoxin inhibits protein synthesis. The cytotoxin may play a role in the local damage to intestinal mucosa that results in

enteric symptoms and inflammatory diarrhea and is consistent with previous finding suggesting that cytotoxin production in shigellosis is related to enteric finding such as fecal leukocytes and occult blood in stool. (Pardo et al., 1986).

The pathogensis and invasiveness of S. typhi may be related to other non cytoxic factors such as envelop virulence antigen (Vi). It has been suggested that direct contact of the Salmonella toxin with host cells causes damage and perhaps promotes invasion. Thus relatively low amounts of cytotoxin may have a role in virulence. S. typhi produced the lowest amount of cytotoxin (Ashkenazi et al., 1988).

In this study the isolated S. typhi showed invasive power as demonstrated on infection of Henle 407 human intestinal cell lines. This might confirm with invasion role in the pathogenesis of S. typhi. Also, two S. typhi strains in our study produce some degree of cytotoxicity as shown morphologically in Henle 407 cell lines and this coincides with cytotoxin role in pathogenesis of enteric fever.

The Vi antigen of S. typhi (group D) enhances the virulence of the organism and enables it to survive in the blood stream. The Vi capsular polysaccharide is both a virulence factor and a protective antigen of S. typhi, its pathogenesis role for S. paratyphi C is less understood (Daniels, et al., 1989).

S. typhi, the causative agent of typhoid fever, must invade the human gastrointestinal tract and multiply within the host to cause disease. Penetration of the epithelial mucosa is a key virulence mechanism and the first major step in the disease process of many

pathogenic bacteria. Several genera of enteric bacteria, including Salmonella, shigella, Escherichia, and yersinia species, have been shown to invade human epithelial cells. S. typhi, the causative agents of human typhoid fever, penetrates the intestinal mucosa and eventually spreads throughout the reticuloendothelial system- Yersinia, shigella and Salmonella species use analogous but genetically different systems to invade epithelial cells (Elsinghorst, et al., 1989).

The ability of Salmonella strains to enter mammalian cells in vitro also appears to correlate with their ability to invade the ileal mucosa in vivo. The growth in tissue culture medium alone results in induction of bacterial invasiveness, after log phase the bacteria grew in tissue culture medium overlying the epithelial cell monolayer and thus because invasive (Lee and Falkow, 1990).

A key pathogenic mechanism of Salmonella is their ablility to invade the cell of the intestinal epithelium. Electron microscopic studies of Salmonella infected laboratory animals and cultured cells have shown that these organisms enter epithelial cells after transient disruption of their surface microvilli. Large number of different genetic loci affect the ability of Salmonella strains to enter cultured cells.

Three Salmonella species (S. typhi, S. choleraesuis, and S. enteritidis) were tested for their ability to invade tissue culture cells. The invasion values ranged from 1 to 30% for S. typhi (Galan and Curtiss III, 1991).

In our study the invasive power of S. typhi strain ranged from 1 to 12% and this is in agreement with previous studies and confirm the role of invasiveness role in pathogenesis of S. typhi.