11 ELISA-DETECTED ST-PRODUCING E.COLI ARE A COMMON CAUSE OF ACUTE-ONSET DIARRHEA IN ITALIAN CHILDREN.

Guarino A., Alessio M., Tarallo L., Fontana M.*, Iacono G.*, Gobio Casali L.*, Guandalini S. Dept.of Pediatrics, Univ. of Naples,Italy
*National Study Group on Acute Diarrheas, Italian Soc. Pediatrics.

ST-producing E.Coli (STEC) is among the commonest cause of childhood diarrhea in developing Countries, whereas in industrialized Countries it is generally considered responsible only for traveler's diarrhea. Our aims were to investigate 1) the incidence of STEC diarrhea in Italy; 2) its clinical features and 3) the suitability of an ELISA method as a diagnostic tool for ST. We have screened 569 children (mean age 43 months, range 0-180 months) with acute-onset diarrhea from 5 Italian towns for STEC and compared an ELISA method (1) with the standard suckling mouse assay (SMA). This is the largest series of children screened for STEC in an industrialized Countries. Results: STEC were detected from 31 children (5.4%): 26 were positive at both tests, 4 only at the SMA, 1 only at the ELISA. Compared to the SMA, the sensitivity of the ELISA was 87%. Specificity was 99.8%. Mean age of children harbouring STEC was 22 months, range 0-62 months ($p<0.05$ vs mean age of the remaining 538 children). Main clinical features were dehydration (23%), fever (29%), vomiting (13%), abdominal pain (35%), "cold" symptoms (29%). Fecal osmolality and osmolar gap were consistent with secretory diarrhea in 80% of children. Mean duration of diarrhea was 5 days. In 3 cases diarrhea lasted more than 14 days. All but one patients did well with oral rehydration therapy: 1 required parenteral rehydration. In conclusion, our data show that in Italy: 1) STEC must be considered as common pathogens in children; 2) STEC cause diarrhea more often in younger children; 3) clinical features are that of a mild to moderate secretory diarrhea. Finally we showed that the ELISA test is reliable and suitable to screen large numbers of strains. 1) J.Clin.Microbiol. 20: 59, 1984.

12 HEAT-STABLE ENTEROTOXIN (ST) ENCODED ON TRANSMISSABLE PLASMID.

A. Guarino, M. Alessio, L. Tarallo, M. Thompson*, R.A. Giannella*.

Dept. of Pediatrics, 2nd School of Medicine, University of Naples, Italy.
*Division of Digestive Disease, University of Cincinnati, Ohio, U.S.A.

E.Coli STs are small peptides encoded on plasmids, detectable by the suckling mouse assay (SMA). We have previously reported the production of an enterotoxin produced by Citrobacter freundii (Cf), whose physico-chemical and biological characteristics are similar to E.Coli STa. In order to investigate whether this toxin might be structurally related to E.Coli STa we developed a rapid, small scale purification scheme and determined the primary sequence of the purified toxin. This procedure is a sequential chromatography on XAD resin followed by reverse phase separation on C-18 silica cartridges and a C-18 silica analytical HPLC column using a methanolic buffer system and a final run in acetonitrile. The recovery of the toxin was monitored at each step by an Elisa method. HPLC-purified Cf toxin: 1) was active in the SMA; 2) competed with pure 18 aminoacid E.Coli STa in the Elisa and 3) comigrated with this in analytical reverse phase chromatography. Sequence analysis of the toxin revealed that Cf ST is an octadecapeptide consisting of the identical aminoacid sequence of the 18 aminoacid STa produced by E.Coli. These data raised the possibility that the organisms may trade plasmids. To ascertain if plasmids encoding ST could be transferred between different bacterial species, ST positive Cf culture was incubated with ST negative, Rifampicin resistant E.Coli. After 24 hours culture was plated onto XLD + Rifampicin. E.Coli strains grown had acquired the ability to produce ST. In conclusion: Cf ST is identical to E.Coli 18 aminoacid STa. The ability of producing STa may be transferred from a species to another through conjugation.

13 HEMOLYTIC E. COLI : A CAUSE OF RECTAL BLEEDING IN INFANTS.

J-L. Gaillard*, G. Cheron, J-F. Mougenot, J-P. Deslys*, C. Nezeloff, J. Schmitz. Departments of Bacteriology* and Paediatrics, Hopital des Enfants Malades, 75015 PARIS.

Rectal bleeding in neonates may be a symptom of necrotizing enterocolitis; however, when isolated, it is often related to ecchymotic stripes or patches on an otherwise normal rectosigmoid mucosa and has a benign evolution (DUPONT C, J Pediatr Gastroenterol Nutr 1987 ; 6 : 257-64). The etiology of this "ecchymotic colitis" is unknown. We report here 3 infants in which hemolytic E. Coli was found to be the cause of such a colitis.

G (♂), A & M (2 ♀) were born between 33 and 40 w of gestation, weighing 2,050 to 2,5 kg. Isolated rectal bleeding occured between d 13 and d 45. Rectosigmoidoscopy showed the caracteristic ecchymotic stripes. Clinical course was spontaneously favorable in 2 ; however oral feeding had to be stopped from d 24 to d 26 in M and could then be resumed without trouble.

Only an hemolytic E. Coli was isolated from cultures of mucosa of G, the histology of which showed bacteria and pneumatosis. An hemolytic E. Coli was also found in pure culture in the stools of A but not of G and M whose mucosae showed only unspecific inflammatory infiltrates. By immunoblotting, sera of the 3 infants were found to strongly recognize a major outer membrane protein of the E. Coli isolated from G and A. Sera of 3 age matched infants and of the mothers were negative. The strains isolated from G and A were negative for the known pathogenic categories of E. Coli (including E. Coli O157:H7).

This is the first report of 1) hemolytic E. Coli being pathogenic in the GI tract, and of 2) a pathogen found responsible for ecchymotic colitis, a condition associated with rectal bleeding in infants.

14 ADHESION OF YERSINIA ENTEROCOLITICA TO HELA CELLS AND RABBIT SMALL INTESTINE.

A. Paerregaard, F. Espersen, J. Hannover Larsen, N. Høiby. Statens Seruminstitut, Department of Clinical Microbiology, 8223, Rigshospitalet, Copenhagen, Denmark.

The presence of a 40-50 megadalton plasmid is a prerequisite for the expression of virulence of Yersinia enterocolitica. Both chromosomal and plasmid genes seem to be involved in controling the interaction between host cells and the bacterium. In this study, the adhesive properties of Y. enterocolitica were studied by means of two different in vitro assay systems. Adhesion to human epithelial cells was measured by direct microscopy of HeLa cells cultivated on coverslips and incubated with bacteria at 37°C. The capability to adhere to the small intestine of rabbits was evaluated by incubation of small disks of intestine with 3H-labeled bacteria at 37°C, followed by washing and finally determination of the retained, intestinal-associated radioactivity by means of liquid scintilation. The bacterial strains studied were Y. enterocolitica serotype O:3, harbouring the virulence plasmid (Ye03P+), its isogenic plasmidless derivate (Ye03P-), and the non-pathogenic plasmidless serotype O:7 (Ye07P-). Ye03P+ as well as Ye03P- adhered extensively to HeLa cells; Ye07P- did not adhere at all. However, while adherence of Ye03P+ to disks of intestinal tissue was considerable, Ye03P- and Ye07P- both adhered to only a small degree. Thus, this study demonstrates that even if pathogenic strains of Y. enterocolitica are capable of adhering to HeLa cells by means of chromosomal genes alone, plasmid encoded properties are necessary for adhesion to the intact mucosal surface, indicating that the binding to cell culture lines differ from the binding to intestine.

15 ANTIGEN ABSORPTION IN CHILDREN WITH COW'S MILK PROTEIN INTOLERANCE.

M. Heyman, E. Grasset, R. Ducroc, J.F. Desjeux. INSERM U.290, Hôpital Saint-Lazare - 75010 Paris, France.

Intestinal absorption of exogenous proteins was studied in infants with cow's milk protein intolerance (CMPI) during the initial period of diagnosis, at the age of 3 ± 1 months and one year later just before and after a milk challenge. Peroral biopsies were mounted in an Ussing chamber for simultaneous measurement of mucosal to serosal transport of Horseradish peroxidase (HRP) in its intact and degraded forms and electrical parameters including short circuit current (Isc) and conductance (G). No modification in HRP absorption was noted in healthy children aged from 2 months to 11 years indicating that gut closure to non-immunoglobulin proteins probably occurred earlier in life. During the initial period of CMPI, transepithelial HRP fluxes rose significantly (J intact HRP = 48.5 ± 15.2 versus 5.9 ± 1.5 pmoles.h^{-1}.cm^{-2} in control children and J degraded HRP = 471 ± 236 versus 37.5 ± 7.7 pmoles.h^{-1}.cm^{-2}), Isc was increased but G unchanged. After several months on a milk free diet, J HRP and Isc returned to control values. Just after the late milk challenge and independently of the clinical issue, J HRP and Isc remained within control values. These results suggest that increased permeability to proteins is probably not constitutive in CMPI but rather secondary to an abnormal immunological response to cow's milk proteins.

16 MACROMOLECULAR ABSORPTION IN INFANTS WITH INFANTILE COLIC.

Lindberg T.*, Lothe L., Jakobsson I.
Department of Pediatrics, University of Lund, Malmö and University of Umeå*, Sweden.

By using a radioimmunological method (1) we have found that the intestinal absorption of the macromolecule human α-lactalbumin (LA) is increased in the immature gut and in infants with gastrointestinal disorders, as cow's milk allergy.
The aim of this study was to measure macromolecule absorption in breast fed and formula fed infants with infantile colic. All infants were born at term. Serum samples were analyzed for LA at 30 and 60 minutes after an intake of human milk. Breast fed infants with infantile colic had increased absorption of LA (mean value 510 µg LA/l serum/l human milk/kg body weight, n=40) compared with breast fed, control infants (mean value 64, n=15). Formula fed infants with infantile colic had higher concentrations of serum LA (mean value 230, n=24) than formula fed control infants (mean value 20, n=12).
Conclusion: Infants with infantile colic have increased absorption of macromolecules (human LA). This is most pronounced in breast fed infants. The finding might suggest that the gut mucosa in some way is affected in infantile colic.

1) Jakobsson et al: Gut 27:1029-34, 1986.