

**UNIVERSIDAD SAN FRANCISCO DE QUITO**

**Colegio de Postgrados**

**High prevalence of Enteroinvasive *Escherichia coli* isolated in a  
region of northern coastal Ecuador**

**Nadia Alejandra Vieira Castañeda**

Tesis de grado presentada como requisito para la obtención del título de Maestría en  
Microbiología

Quito, noviembre del año 2006

**Universidad San Francisco de Quito**

**Colegio de postgrados**

**HOJA DE APROBACIÓN DE TESIS**

**High prevalence of Enteroinvasive *Escherichia coli* isolated in a region of northern coastal Ecuador**

**Nadia Alejandra Vieira Castañeda**

Gabriel Trueba, Ph.D.  
Director de la Maestría en Microbiología y  
Director de la Tesis

-----

William Cevallos, M.D, M.Sc.  
Miembro del Comité de Tesis

-----

Venancio Arahana, Ph.D.  
Miembro del Comité de Tesis

-----

Hugo Valdebenito, Ph.D.  
Decano del Colegio de  
Ciencias Biológicas y Ambientales

-----

Víctor Viteri, Ph. D.  
Decano del Colegio de Postgrados

-----

Quito, noviembre del año 2006

© Derechos de autor  
Nadia Alejandra Vieira Castañeda  
2006



## ABSTRACT

**Background:** Although Enteroinvasive *E. coli* (EIEC) is a clinically important pathogen, few clinical and community studies have specifically looked for EIEC, compared to Shigellae or other diarrhea causing *E. coli*. Due to the lack of scientific attention in studies of diarrheal disease, the epidemiology of EIEC is poorly understood, and the degree to which EIEC is being mis-diagnosed as Shigellae is unknown.

**Methods:** *E. coli* and Shigellae were identified in a case-control study in northern coastal Ecuador. Infection was assessed by PCR specific for *LT* and *STa* genes of enterotoxigenic *E. coli* (ETEC), the *bfp* gene of enteropathogenic *E. coli* (EPEC), and the *ipaH* gene of both enteroinvasive *E. coli* and Shigellae. Distinction between EIEC and Shigellae was achieved with biochemical tests

**Results:** The pathogenic *E. coli* most frequently identified were EIEC (3.2 cases / 100 persons) and Shigellae (1.5 cases / 100 persons), followed by ETEC (1.3 cases / 100 persons), and EPEC (0.9 cases / 100 persons). EIEC exhibited similar risk factor relationships with other pathotypes analyzed but different age-specific infection rates.

**Conclusions:** Data from this study suggest that the attributable burden of EIEC infection may be underestimated. Given the potential importance of this invasive pathogen, more work should be focused on how EIEC transmission patterns may be similar and/or different from that of Shigellae to improve our basic understanding of EIEC epidemiology, including individual risk factors, and possible sources of infection.

## RESUMEN

**Generalidades:** Aunque la *E. coli* Enteroinvasiva (EIEC) es un patógeno clínicamente importante, existen pocos estudios clínicos que ha buscado específicamente a EIEC comparando con Shigellae y otras *E. coli* patogénicas. Es por esto que la epidemiología de EIEC es poco entendida y el grado de confusión con Shigellae es desconocido.

**Métodos:** En este estudio de caso-control en la costa norte del Ecuador fueron analizadas *E. coli* patogénicas y Shigellae. Mediante PCR específico para los genes *LT* y *STa* se identificaron a las *E. coli* enterotoxigenicas (ETEC), el gen *bfp* para las *E. coli* enteropatógenicas (EPEC) y el gen *ipaH* para identificar a Shigellae y *E. coli* enteroinvasiva (EIEC). La distinción entre EIEC y Shigella se realizó mediante pruebas bioquímicas.

**Resultados:** De todas las *E. coli* patogénicas aisladas, la más frecuentemente fue EIEC (3.2 casos/100 personas) y Shigellae (1.5 casos /100 personas), seguidas de ETEC (1.3 casos/100 personas), y EPEC (0.9 casos/100 personas). EIEC mostró un factor de riesgo similar al de Shigellae pero con diferente tasa de infección por edad

**Conclusiones:** Los resultados de este estudio sugieren que el riesgo de infección atribuido generalmente a EIEC podría ser mayor al estimado. Considerando la importancia potencial de este patógeno invasivo, pensamos que se debería poner mayor énfasis en la descripción de sus patrones de transmisión y entender las relaciones epidemiológicas con *E. coli* patotipe Shigellae.

## CONTENTS

BACKGROUND.....	¡Error! Marcador no definido.
PARTICIPANTS, MATERIALS, AND METHODS.....	¡Error! Marcador no definido.
<b>definido.</b>	
Study population .....	¡Error! Marcador no definido.
Study design .....	¡Error! Marcador no definido.
Pathogenic <i>E. coli</i> identification.....	¡Error! Marcador no definido.
PFGE typing.....	¡Error! Marcador no definido.
Invasion Cell Assay.....	¡Error! Marcador no definido.
Statistical Analysis .....	¡Error! Marcador no definido.
RESULTS.....	¡Error! Marcador no definido.
Detection and prevalence of pathogenic <i>E. coli</i> .....	¡Error! Marcador no definido.
<b>definido.</b>	
Geographic distribution of pathotypes.....	¡Error! Marcador no definido.
Age distribution of pathotypes .....	¡Error! Marcador no definido.
Risk factor analysis .....	¡Error! Marcador no definido.
Pulsed Field Gel Electrophoresis (PFGE) typing.....	¡Error! Marcador no definido.
<b>definido.</b>	
DISCUSSION .....	¡Error! Marcador no definido.
REFERENCES .....	¡Error! Marcador no definido.
FIGURES AND TABLES .....	¡Error! Marcador no definido.

## FIGURES AND TABLES

Figure 1. Xbal PFGE patterns.....

**¡Error! Marcador no definido.**

Table 1. Isolation of *E. coli* pathotypes and *Shigellae* by geographic region and case (D+) versus control (D-) status.....

**¡Error! Marcador no definido.**

Table 2. Isolation of *E. coli* pathotypes and *Shigellae* by age.....

**¡Error! Marcador no definido.**

Table 3. Bivariate analysis aggregating infections of *E. coli* pathotypes and *Shigellae*.....

**¡Error! Marcador no definido.**

Table 4. Bivariate analysis by *E. coli* pathotypes and *Shigellae*.....

**¡Error! Marcador no definido.**

## BACKGROUND

Enteroinvasive *Escherichia coli* (EIEC) was first shown to cause diarrheal disease in otherwise healthy volunteers in 1971 by DuPont et al. [1]. It is known to cause shigellosis-like symptoms in both adults and children. Despite its acknowledged status as a human pathogen, very little research has been conducted to identify individual risk factors for infection, possible reservoirs, or even infection rates. Other pathotypes, such as enteropathogenic (EPEC), enterohemorrhagic (EHEC), and enterotoxigenic (ETEC) *E. coli*, as well as *Shigellae*, have received much more scientific attention.

A potential contributor to the lack of attention to the epidemiology of EIEC is that it is often observed to be an infrequent cause of diarrhea relative to other diarrhea-causing



*E. coli*. In a Medline search of studies testing for the presence of EIEC, we identified 42 articles. Of these studies, 35% (15) found no EIEC and 40% (17) found EIEC to be a minor strain [2-18]; *i.e.*, representing less than 4% and fewer than 10 isolated cases and of the collected stool samples. There were, however, notable exceptions. In 1989, Kain et al. [19] identified 15 EIEC samples in 221 childhood diarrhea cases in a Beijing hospital; in 1985, Taylor et al. [20] observed 17 cases of EIEC in 410 children with diarrhea in a Bangkok hospital; and in 1982-1986 in Chile, Faundez et al. [21] observed 17 EIEC cases in 912 infants with diarrhea. More recently, in the mid-1990s, EIEC was identified in 87 of 1579 stool samples from patients with travel-associated diarrhea [22]. In the late 1990s, 16 EIEC positive isolates were identified from 279 Senegalese individuals [23]; and EIEC was the predominant enteropathogen during a two month period of increased diarrhea episodes in the Jordan Valley [24]. These and four additional studies [25-28] represented all studies identified that reported 10 or more diarrheal cases positive for EIEC (7 of 42, or 22% of studies reviewed). These studies are widely distributed geographically, including Europe, Central and South America, the Middle East, western Africa, and southeastern Asia. In over half of the studies that isolated EIEC, EIEC was identified as a possible etiologic agent of diarrhea.

Thus, EIEC is seldom identified, and when it is found it tends to be in small numbers. EIEC infection rates have never been reported for Ecuador. We report here EIEC as the predominant *E. coli* pathotype identified from both cases and controls in a community-based case-control study in northern Ecuador. Patterns of EIEC infection

are compared to infections with Shigellae as well as Enterotoxigenic (ETEC) and Enteropathogenic (EPEC) *E. coli*

## **PARTICIPANTS, MATERIALS, AND METHODS**

### **Study population**

The study area is located in the northern Ecuadorian province of Esmeraldas in the cantón Eloy Alfaro, which comprises approximately 150 villages. The study reported here was carried out in 22 communities, all located within the drainage system of three rivers, the Cayapas, Santiago, and Onzole. Borbón is situated at the confluence of the rivers, is the largest of the study communities, and the main population center of the region (pop. approximately 5000). A random sample of 200 households in Borbón was selected and enrolled into the study. In the 21 smaller villages, all households were eligible to be enrolled into the study, and over 98% consented to participate. Four of these villages are located along a road. The remaining 17 villages are primarily accessed by river: two are downstream from Borbón, and 15 are upstream from Borbón. Oral consent for participation was obtained at both the village and household levels. IRB committees at the Universidad San Francisco de Quito, and University of California, Berkeley approved all protocols. Details on the region can be accessed elsewhere [29-31].

### **Study design**

In Borbón, one 15-day-case-control study was conducted during July 2005. Each of the 21 smaller study villages was visited three times between August 2003 and June 2005. During each visit a 15-day case-control study was conducted in

which fecal specimens were collected for every case of diarrhea in the community. For each case, three asymptomatic control specimens were also collected: one from a member of the case's household and two randomly selected from the community. A case was defined as an individual that had three or more loose stools in a 24-hour period. A control was defined as someone with no symptoms of diarrhea.

### **Pathogenic *E. coli* identification**

For each stool sample, five lactose-fermenting colonies were isolated on a MacConkey agar plate. The five colonies were pooled, resuspended in 300  $\mu$ L of sterile distilled water, and boiled for 10 min to release the DNA. The resulting supernatant was used for PCR testing. Identification of *E. coli* pathovars was performed by PCR, with primers designed to amplify the *bfp* gene of EPEC, the *LT* and *Stx* genes of ETEC, and the *ipaH* gene of EIEC [15]. Non-lactose-fermenting colonies that were identified by API 20E (bioMérieux Corp) as Shigellae or *E. coli*, were subsequently analyzed by PCR with primers designed to amplify the *ipaH* gene. The primer sequences and the amplification protocols were previously published by Tornieporth et al. [15]. Briefly, a 2.5  $\mu$ L aliquot of DNA suspension was amplified with PuRe Taq Ready-To-Go™ PCR beads (Amersham Biosciences). The 25  $\mu$ L solution added to the beads contained 0.08  $\mu$ M of each appropriate oligonucleotide primer. The cycling parameters were as follows: 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 2 min, and extension at 72°C for 1 min. The PCR products were resolved by 1.6% agarose gel electrophoresis and visualized by UV transillumination

after ethidium bromide staining. Positive and negative control strains for PCR tests were kindly provided by Lee W. Riley, University of California Berkeley.

### **PFGE typing**

All of the *E. coli* isolates identified by PCR as EIEC or Shigellae were subjected to Pulsed Field Gel Electrophoresis typing (PFGE). Briefly, an overnight cell culture was resuspended in SE buffer (75 mM NaCl, 25 mM EDTA, pH 8.0) to an OD<sub>610</sub> of approximately 0.7. An aliquot of 200 µL of this suspension was mixed with an equal volume of a solution containing 10 µL of proteinase K (20 mg/mL), 1% SDS, and 1% agarose (Pulse Field Certified Agarose, Bio-Rad Laboratories). This mixture was dispensed into disposable plug molds. After solidification, the agarose plugs were transferred to tubes containing 1.5 mL of lysis buffer (50mM Tris-HCl pH 8.0, 50mM EDTA pH 8.0, 1% *N*-laurylsarcosine, and 0.1 mg/mL of proteinase K) and lysis was carried out overnight at 54°C with constant shaking. After lysis, the agarose plugs were washed five times with 10 mL of warm (50°C) TE buffer (10 mM Tris, 1 mM EDTA pH 8.0) and stored in TE at 4°C. Slices of the agarose plugs were digested with 60 U of *Xba*I (New England Biolabs) overnight at 37°C, in accordance with the manufacturer's instructions.

Restriction fragments were separated in a 1% Pulse Field Certified Agarose (Bio-Rad Laboratories) gel by PFGE using a CHEF Electrophoresis Cell (Bio-Rad Laboratories). The gels were run in 0.5X TBE buffer with 100 µM thiourea [32]. Running conditions were 14°C at 6 V/cm, with an initial pulse time of 2.2 s that was increased to 54.2 s

over the course of 23 h. Gels were stained with ethidium bromide and visualized with UV light.

Images of PFGE electrophoretic patterns were imported and analyzed with GelCompar II, version 2.0 (Applied Maths, Kortrijk, Belgium). From the electrophoretic curves, a distance matrix was calculated using the Pearson correlation algorithm implemented by the GelCompar program. A dendrogram was generated from the distance matrix by the neighbor-joining method. The PFGE fingerprint patterns of isolates that appeared to cluster together on the dendrograms were then visually examined to confirm their identity.

### Invasion Cell Assay

The invasive phenotype of bacterial isolates was confirmed by inoculation of a confluent monolayer of HeLa cells. Isolates were grown in LB media to an  $OD_{600}$  of 0.4-0.6, washed twice with sterile PBS, and then resuspended to an  $OD_{600}$  of 0.5. 25  $\mu$ L of this suspension was added to PBS-washed HeLa cells and 1 mL DMEM. This mixture was centrifuged, incubated in 5%  $CO_2$  at 37° C for three hours. Cells were washed three times with PBS, and then incubated at 37° C for one hour with 1.5 mL of DMEM containing 100  $\mu$ L/mL gentamycin. Cells were lysed by pipetting after the addition of 1 mL 0.1% TritonX-100 in PBS and shaking for 5 min. Serial dilutions were plated to LB media and grown overnight at 37° C. Colonies were counted and bacteria were visualized microscopically with Giemsa stain.

## **Statistical Analysis**

All data were analyzed with Stata 8.0 (Stata Corp.). Prevalence of infection was estimated as a weighted sum of cases and controls assuming that all cases were identified during each 15-day visit to a community and that the controls were randomly sampled. Because wage income is relatively uncommon in the study area, socioeconomic status was assessed through ownership of material goods. Surveys were conducted in each case and control household to determine the number and type of consumer goods each household possessed, and a standard living index (SLI) was calculated by weighting and summing these results. Sanitation was defined as either improved (pit latrine or septic tank) or unimproved (river or open ground), water source as improved (well or piped) or unimproved (surface). Food consumption habits were reported for the week prior to stool collection.

## RESULTS

### **Detection and prevalence of pathogenic *E. coli***

A total of 4220 individuals from 21 villages and 877 from Borbón were enrolled in this study. Between August 2003 and July 2005, 342 cases of diarrhea were identified and 970 asymptomatic controls were selected (three for each diarrhea sample). From these cases and controls, 915 stool samples (236 cases, 679 controls) were subjected to further analysis.

Lactose-fermenting enterobacterial colonies were evaluated by PCR from all 915 stool samples. Non-lactose fermenting colonies were also isolated from 355 fecal samples and evaluated by PCR. Forty-three of these isolates were identified as EIEC (21 cases, 21 controls, one unknown). Seven isolates were lactose fermenters (lac +) and further identified by PCR to contain the *ipaH* gene. Thirty-six isolates were lactose non-fermenters (lac -) that were further identified by biochemical tests as *E. coli* and by PCR to contain the *ipaH* gene. A random sample of 10 of these isolates (five cases, four controls, one unknown) was further analyzed by a tissue culture invasion assay, and 80% (five cases, two controls, one unknown) were confirmed to be invasive.



Ninety-one pathogenic *E. coli* strains or Shigellae were identified in 88 samples. Of the three co-infections, one individual was co-infected with EIEC and Shigellae, one with ETEC and EIEC, and the other with EPEC and Shigellae. The prevalence of each pathotype stratified by location and case versus control status is shown in Table 1. The pathogenic bacteria most frequently identified were EIEC (3.2 cases / 100 persons) and Shigellae (1.5 cases / 100 persons), followed by ETEC (1.3 cases / 100 persons), and EPEC (0.9 cases / 100 persons).

### **Geographic distribution of pathotypes**

All pathotypes had a higher prevalence in Borbón than in the smaller communities. In Borbón, EIEC was the dominant pathotype for both cases and controls (21 and 13 cases/100 persons respectively). In the communities, EIEC and ETEC were the dominant pathotypes in the diarrhea cases (6.3 and 7.3 cases/100 persons respectively). In the community controls, however, the prevalence of all pathotypes was approximately one case/100 persons.

Only EIEC and ETEC infections were significantly associated with diarrheal disease in the communities. Though prevalence of infection was higher in Borbón than in the communities, infection was not significantly associated with disease in Borbón for any of the pathotypes.

### **Age distribution of pathotypes**

In general, prevalence dropped off in the > 20 year age group (Table 2). This was less evident in EIEC (RR = 1.5 comparing 0 – 5 year olds with those > 20 years old; 95% CI: 1.1, 2.0) than in Shigellae (RR = 3.1; 95% CI: 2.1, 4.5), EPEC (RR = 2.9; 95% CI: 2.1, 4.1) and ETEC (RR = 3.7; 95% CI: 2.4, 5.6). Specifically, eight EIEC infections (2.5 cases/100 persons) were identified in the > 20 year old age group, seven of which were asymptomatic individuals. In contrast the prevalence of ETEC and Shigellae in the > 20 year age group was 0.4 and 0.7 cases/100 persons respectively.

### **Risk factor analysis**

Aggregating all *E. coli* and Shigellae isolates showed no association between infection and water source, sanitation, or food consumption (Table 3). The Standard Living Index (SLI) was protective for infection (RR = 0.91; 95% CI: 0.86, 0.97). Living with an infected case did not pose a significant risk of asymptomatic infection (OR = 2.2; 95% CI: 0.5, 8.1), although it did have the highest point estimate. These results were generally consistent across all *E. coli* pathotypes and Shigellae though not always statistically significant, possibly due to small sample size (Table 4).

### **Pulsed Field Gel Electrophoresis (PFGE) typing**

PFGE analysis of the 43 EIEC isolates identified one five-member cluster, one three-member cluster, and five two-member clusters (Figure 1). Four of the five two-member clusters were within-village clusters, three of them within Borbón. All other

clusters linked individuals in different villages: one connected a road community with Borbón; one connected a Santiago river community with Borbón, and one connected communities along two river basins (the Santiago and the Onzole), as well as a community downstream from Borbón. These clusters represent 18 of 43 isolates. The remaining 25 isolates were not related to each other or to any of the seven clusters, suggesting that the presence of EIEC is likely due to the appearance of multiple clones in the communities rather than to a single source outbreak

## DISCUSSION

The high prevalence levels of EIEC infection in diarrheal cases observed in this study (20.5 cases / 100 persons in Borbón and 6.3 cases / 100 persons in the communities) are unprecedented in both hospital- or community-based studies of *E. coli*. EIEC was isolated two to three times more often than ETEC, EPEC, and Shigellae. This result contradicts the opposite finding more commonly seen in the literature on pathogenic *E. coli*. For example, Valentiner-Branth et al. [17] estimated an ETEC incidence of 2.45 cases / child-year vs. an EIEC incidence of 0.29 cases/child-year in Ghana. Of the studies reviewed in the introduction, 75% found few or no EIEC infections. The two most notable exceptions were a 1997 travelers' diarrhea study in which 6% of the stool samples analyzed detected EIEC [22] and a 1989 study in Beijing in which 7% of the stool samples from children with diarrhea were positive for EIEC.

There is no clear explanation for the elevated prevalence of EIEC relative to other pathotypes in this region of Ecuador. One possibility would be that the EIEC isolates were from a single point-source outbreak. A number of EIEC outbreaks have been reported [33-37], many of which were food-borne [38-41]. Previous studies have shown the potential for using molecular tools to identify EIEC outbreak clusters [42, 43]. Our PFGE results, however, indicate that these EIEC isolates were not from any single source; *i.e.*, there were clearly multiple sources of EIEC within our study region. Interestingly, the larger cluster (A) shown in Figure 1 suggests that transmission occurred between two river basins (the Santiago and the Onzole), as well as downstream from Borbón. The most likely explanation is that the source was Borbón. The lack of evidence for a single source of infection suggests that there may be

specific risk factors that promote the transmission of EIEC. Unfortunately, very little is known about the epidemiology of EIEC. Our small sample size precluded us from making any firm conclusions about the risks associated with the 43 identified infections. After aggregating all *E. coli* infections (Table 3), higher socioeconomic status (SES) was found to be protective (OR = 0.91 [0.86, 0.97]). There were no other significant associations with regard to water, sanitation or food risks. When disaggregated, the protective relationship with SES was maintained with EIEC and Shigellae, but was no longer significant with ETEC and EPEC (Table 4). Additional samples are needed to improve our understanding of EIEC transmission patterns within the region.

A number of methodological issues exist that may partially explain why low levels of EIEC are found in other studies. Specifically, microbiological analyses used in other studies often do not distinguish between EIEC and Shigellae (see for example [9, 17, 21, 44]). Differentiating between EIEC and Shigellae is difficult due to their genetic similarities. The four species of Shigellae are often considered to be a type of *E. coli*, and are most similarly related to EIEC [45]; these bacteria are characterized by a large virulence plasmid of 220kb and their ability to invade epithelial cells and disseminate from cell to cell [45]. Depending on the design of the particular study this may result in underestimates of EIEC or of Shigellae [64].

Another methodological issue is that EIEC can be either lactose-positive or negative [46], an unusual trait among *E. coli*. In this study 36 EIEC isolates were lactose-negative and only seven were lactose-positive. Many other studies only screen for

lactose-positive *E. coli* strains (see for example [4, 6, 12, 16, 20, 47]), and thus may underestimate the prevalence of EIEC infection.

From a clinical perspective, distinguishing between these two bacteria is unnecessary since treatment of the two infections is the same. From a public health intervention perspective, however, the distinction may be more important. Although these organisms are closely related, EIEC and Shigellae have important differences relating to transmission. The minimum infectious inoculum of EIEC is higher than at least two of the four Shigellae species [48, 49], and some studies suggest that the mode of transmission may differ. Shigellae has primarily been associated with transmission via personal contact [50], whereas EIEC has principally been associated with contaminated food and water [33, 38], though cases of person-to-person transmission of EIEC have been noted [34].

In our study, there was some evidence of the similarity and divergence between the epidemiology of EIEC and Shigellae. For example, both exhibited a similar relationship to SES levels and to crowding, and both exhibited insignificant relationships with water source, sanitation level, and food consumption.

In contrast, with respect to age-stratified infection rates, EIEC was found more often in older age groups than was Shigellae, suggesting that adults were exposed to EIEC more than to Shigellae. The EIEC isolated from adults was more likely to be from controls, suggesting that EIEC is highly endemic; *i.e.*, exposure occurs in younger age groups, resulting in immune adults. These analyses, however, are limited due to the small sample size.

Given the potential importance of this invasive pathogen, more work should be focused on why EIEC is highly prevalent in this study region. In addition, studies that properly distinguish EIEC from Shigellae would help determine if this high prevalence is also common in other areas of the world. Additional studies that provide samples representative of communities would provide valuable information on the EIEC epidemiology patterns. Understanding these patterns of EIEC infection and transmission would provide important information on how best to design intervention and control strategies targeted at both EIEC and Shigellae.

## **REFERENCES**

1. DuPont HL, Formal SB, Hornick RB, et al. Pathogenesis of *Escherichia coli* diarrhea. N Engl J Med 1971;285:1-9
2. Akinyemi KO, Oyefolu AO, Opere B, Otunba-Payne VA and Oworu AO. *Escherichia coli* in patients with acute gastroenteritis in Lagos, Nigeria. East Afr Med J 1998;75:512-5
3. Katouli M, Jaafari A, Farhoudi-Moghaddam AA and Ketabi GR. Aetiological studies of diarrhoeal diseases in infants and young children in Iran. J Trop Med Hyg 1990;93:22-7
4. Kim KH, Suh IS, Kim JM, Kim CW and Cho YJ. Etiology of childhood diarrhea in Korea. J Clin Microbiol 1989;27:1192-6
5. Lee CL, Chiou SI, Liu TP and Pan TM. [Pathogenic strains of *Escherichia coli* in Taiwan]. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi 1997;30:55-9
6. Okeke IN, Lamikanra A, Steinruck H and Kaper JB. Characterization of *Escherichia coli* strains from cases of childhood diarrhea in provincial southwestern Nigeria. J Clin Microbiol 2000;38:7-12
7. Orlandi PP, Silva T, Magalhaes GF, et al. Enteropathogens associated with diarrheal disease in infants of poor urban areas of Porto Velho, Rondonia: a preliminary study. Mem Inst Oswaldo Cruz 2001;96:621-5
8. Pabst WL, Altwegg M, Kind C, Mirjanic S, Hardegger D and Nadal D. Prevalence of enteroaggregative *Escherichia coli* among children with and without diarrhea in Switzerland. J Clin Microbiol 2003;41:2289-93
9. Rappelli P, Folgosa E, Solinas ML, et al. Pathogenic enteric *Escherichia coli* in children with and without diarrhea in Maputo, Mozambique. FEMS Immunol Med Microbiol 2005;43:67-72
10. Regua-Mangia AH, Gomes TA, Vieira MA, Andrade JR, Irino K and Teixeira LM. Frequency and characteristics of diarrhoeagenic *Escherichia coli* strains isolated from children with and without diarrhoea in Rio de Janeiro, Brazil. J Infect 2004;48:161-7



11. Shehabi AA, Bulos NK and Hajjaj KG. Characterization of diarrhoeagenic *Escherichia coli* isolates in Jordanian children. Scand J Infect Dis 2003;35:368-71
12. Sunthadvanich R, Chiewsilp D, Seriwatana J, Sakazaki R and Echeverria P. Nationwide surveillance program to identify diarrhea-causing *Escherichia coli* in children in Thailand. J Clin Microbiol 1990;28:469-72
13. Taylor DN, Houston R, Shlim DR, Bhaibulaya M, Ungar BL and Echeverria P. Etiology of diarrhea among travelers and foreign residents in Nepal. Jama 1988;260:1245-8
14. Teng LJ, Hsueh PR, Liaw SJ, Ho SW and Tsai JC. Genetic detection of diarrheagenic *Escherichia coli* isolated from children with sporadic diarrhea. J Microbiol Immunol Infect 2004;37:327-34
15. Tornieporth NG, John J, Salgado K, et al. Differentiation of pathogenic *Escherichia coli* strains in Brazilian children by PCR. J Clin Microbiol 1995;33:1371-4.
16. Torres ME, Pirez MC, Schelotto F, et al. Etiology of children's diarrhea in Montevideo, Uruguay: associated pathogens and unusual isolates. J Clin Microbiol 2001;39:2134-9
17. Valentiner-Branth P, Steinsland H, Fischer TK, et al. Cohort study of Guinean children: incidence, pathogenicity, conferred protection, and attributable risk for enteropathogens during the first 2 years of life. J Clin Microbiol 2003;41:4238-45
18. Youssef M, Shurman A, Bougnoux M, Rawashdeh M, Bretagne S and Strockbine N. Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. FEMS Immunol Med Microbiol 2000;28:257-63
19. Kain KC, Barteluk RL, Kelly MT, et al. Etiology of childhood diarrhea in Beijing, China. J Clin Microbiol 1991;29:90-5
20. Taylor DN, Echeverria P, Sethabutr O, et al. Clinical and microbiologic features of Shigella and enteroinvasive *Escherichia coli* infections detected by DNA hybridization. J Clin Microbiol 1988;26:1362-6

21. Faundez G, Figueroa G, Troncoso M and Cabello FC. Characterization of enteroinvasive *Escherichia coli* strains isolated from children with diarrhea in Chile. *J Clin Microbiol* 1988;26:928-32
22. Altwegg M, Perschil I and Gruner E. [Molecular biology detection and antibiotic sensitivities of *Shigella* spp. and entero-invasive *Escherichia coli* (EIEC) in patients returning from the tropics]. *Schweiz Rundsch Med Prax* 1997;86:348-51
23. Gassama-Sow A, Sow PS, Gueye M, et al. Characterization of pathogenic *Escherichia coli* in human immunodeficiency virus-related diarrhea in Senegal. *J Infect Dis* 2004;189:75-8
24. Meqdam MM, Youssef MT, Rawashdeh MO and Al-khdour MS. Non-seasonal viral and bacterial episode of diarrhoea in the Jordan Valley, West of Jordan. *FEMS Immunol Med Microbiol* 1997;18:133-8
25. Cortes-Ortiz IA, Rodriguez-Angeles G, Moreno-Escobar EA, Tenorio-Lara JM, Torres-Mazadiego BP and Montiel-Vazquez E. [Outbreak caused by *Escherichia coli* in Chalco, Mexico]. *Salud Publica Mex* 2002;44:297-302
26. Nguyen TV, Le Van P, Le Huy C, Gia KN and Weintraub A. Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. *J Clin Microbiol* 2005;43:755-60
27. Ratchtrachenchai OA, Subpasu S, Hayashi H and Ba-Thein W. Prevalence of childhood diarrhoea-associated *Escherichia coli* in Thailand. *J Med Microbiol* 2004;53:237-43
28. Svenungsson B, Lagergren A, Ekwall E, et al. Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. *Clin Infect Dis* 2000;30:770-8
29. Rival L. The meanings of forest governance in Esmeraldas, Ecuador. *Oxford Development Studies* 2003;31:479-501

30. Sierra R. Traditional resource-use systems and tropical deforestation in a multi-ethnic region in North-west Ecuador. *Environmental Conservation* 1999;26:136-145
31. Whitten NE. *Black frontiersmen; a South American case.* [Cambridge, Mass.]: Schenkman Pub. Co., 1974
32. Zhang Y, Yakrus MA, Graviss EA, et al. Pulsed-field gel electrophoresis study of *Mycobacterium abscessus* isolates previously affected by DNA degradation. *J Clin Microbiol* 2004;42:5582-7
33. Gordillo ME, Reeve GR, Pappas J, Mathewson JJ, DuPont HL and Murray BE. Molecular characterization of strains of enteroinvasive *Escherichia coli* O143, including isolates from a large outbreak in Houston, Texas. *J Clin Microbiol* 1992;30:889-93
34. Harris JR, Mariano J, Wells JG, Payne BJ, Donnell HD and Cohen ML. Person-to-person transmission in an outbreak of enteroinvasive *Escherichia coli*. *Am J Epidemiol* 1985;122:245-52
35. Ozbonfil D, Cohen D, Ohad E and Sechter I. An outbreak of enteritis associated with enteroinvasive *Escherichia coli* in an Israeli military base. *Public Health Rev* 1990;18:171-7
36. She SL. [An outbreak of diarrhoea in newborns caused by EIEC]. *Zhonghua Liu Xing Bing Xue Za Zhi* 1989;10:333-6
37. Sobotkova J, Hausner O, Aldova E, Jiskrova R and Maskova L. [An epidemic of diarrhea caused by enteroinvasive serotypes of *Escherichia coli* O28 ac (Ca 792)]. *Cesk Epidemiol Mikrobiol Imunol* 1984;33:315
38. Marier R, Wells JG, Swanson RC, Dalthan W and Mehlman IJ. An outbreak of enteropathogenic *Escherichia coli* foodborne disease traced to imported french cheese. *Lancet* 1973;ii:1376-1378
39. Snyder JD, Wells JG, Yashuk J, Puhr N and Blake PA. Outbreak of invasive *Escherichia coli* gastroenteritis on a cruise ship. *Am J Trop Med Hyg* 1984;33:281-284

40. Yamamura K, Sumi N, Egashira Y, Fukuoka I, Motomura S and Tsuchida R. [Food poisoning caused by enteroinvasive *Escherichia coli* (O164:H-)--a case in which the causative agent was identified]. *Kansenshogaku Zasshi* 1992;66:761-8
41. Yang SZ. [An outbreak of food poisoning due to entero-invasive *Escherichia coli*]. *Zhonghua Liu Xing Bing Xue Za Zhi* 1986;7:129-31
42. Beutin L, Gleier K, Kontny I, Echeverria P and Scheutz F. Origin and characteristics of enteroinvasive strains of *Escherichia coli* (EIEC) isolated in Germany. *Epidemiol Infect* 1997;118:199-205
43. Llovet T, Coll P, March F, et al. Comparison of macrorestriction analysis of genomic DNA by pulsed-field gel electrophoresis and ribotyping with conventional methods for differentiation of *Escherichia coli* O124 isolates. *Clin Microbiol Infect* 1995;1:127-133
44. Jallat C, Livrelli V, Darfeuille-Michaud A, Rich C and Joly B. *Escherichia coli* strains involved in diarrhea in France: high prevalence and heterogeneity of diffusely adhering strains. *J Clin Microbiol* 1993;31:2031-7
45. Pupo GM, Lan R and Reeves PR. Multiple independent origins of Shigella clones of *Escherichia coli* and convergent evolution of many of their characteristics. *Proc Natl Acad Sci U S A* 2000;97:10567-72
46. Nataro JP, Kaper JB. *Escherichia coli*. *Clin Microbiol Rev* 1998;11:142-201
47. Sarantuya J, Nishi J, Wakimoto N, et al. Typical enteroaggregative *Escherichia coli* is the most prevalent pathotype among *E. coli* strains causing diarrhea in Mongolian children. *J Clin Microbiol* 2004;42:133-9
48. DuPont HL, Levine MM, Hornick RB and Formal SB. Inoculum size in shigellosis and implications for expected mode of transmission. *J Infect Dis* 1989;159:1126-8
49. Hsia RC, Small PL and Bavoil PM. Characterization of virulence genes of enteroinvasive *Escherichia coli* by TnphoA mutagenesis: identification of invX, a gene required for entry into HEp-2 cells. *J Bacteriol* 1993;175:4817-23

50. Niyogi SK. Shigellosis. J Microbiol 2005;43:133-43

**FIGURES AND TABLES**

