



## Echovirus-4 Meningitis Outbreak Imported from India

Federico Gobbi, MD, DTM&H,\*<sup>†</sup> Guido Calleri, MD,<sup>‡</sup> Claudia Spezia, MD,<sup>‡</sup>  
Filippo Lipani, MD,<sup>‡</sup> Rosanna Balbiano, MD,<sup>‡</sup> Maura De Agostini, MD,<sup>‡</sup>  
Maria Grazia Milia, MD,<sup>§</sup> and Pietro Caramello, MD<sup>‡</sup>

\*Centre for Tropical Diseases, S. Cuore Hospital, Negrar, Verona Province, Italy; <sup>†</sup>Department of Prevention, ULSS 20, Verona, Italy; <sup>‡</sup>Unit A of Infectious and Tropical Diseases, Amedeo di Savoia Hospital, Torino, Italy; <sup>§</sup>Laboratory of Microbiology and Virology, Amedeo di Savoia Hospital, Torino, Italy

DOI: 10.1111/j.1708-8305.2009.00375.x

We describe seven cases of meningitis in a group of young Italian travelers coming back from India. Virologic studies identified echovirus-4 as the cause of this cluster of cases, the first imported echovirus outbreak in Italy. Enteroviruses may play an important role in undiagnosed fevers in travelers.

Traveling to tropical regions entails being exposed to a wide range of health risks.<sup>1</sup> Travelers' diarrhoea is the most frequent health problem,<sup>2</sup> but the range of travel-related illnesses also includes potential life-threatening diseases; still, an important percentage of febrile syndromes remain undiagnosed.<sup>3,4</sup>

Human enteroviruses are responsible for a wide spectrum of diseases in all age groups, although infection and illness commonly affect infants and young children.

Transmission occurs predominantly through the oral-fecal route. The incubation period may vary according to the clinical syndrome, being mostly of 3 to 5 days: more than 90% of infections are asymptomatic or result in an undifferentiated febrile illness.

When disease occurs, the spectrum and severity of clinical manifestations vary with age, gender, and immune status of the host; meningitis is by far the most common central nervous system manifestation, generalized and focal encephalitis is less frequent. The most frequently isolated serotypes in Europe are 30, 13, and 6.<sup>5-7</sup>

### Case Report

We describe an outbreak that occurred in Turin (Italy), in September 2006, in a group of 17 young Italian travelers (11 females and 6 males, in an age range of 18–32 years) after spending 2 weeks in Krishnanagar, a town 80 km from Calcutta (India).

**Corresponding Author:** Federico Gobbi, MD, DTM&H, Malattie Tropicali, Ospedale Sacro Cuore, Via don A. Sempredoni 5, I-37024 Negrar, Verona, Italy. E-mail: fedgobbi@tin.it

All were vaccinated for tetanus, hepatitis A and B, typhoid fever i.m.: the prescribed antimalarial chemoprophylaxis was taken regularly by all members of the group.

Between 48 and 72 hours after returning to Italy, eight of them developed the following signs and symptoms: stiff neck (2/8), fever (8/8), headache (8/8), vomiting (1/8), and sore throat (1/8). Seven of them were admitted in our hospital (see Table 1).

Only two patients had a stiff neck but the lumbar puncture, carried out in the first case, showed hypercellularity (1,390 cells, 70% N): for this reason it was also performed on the other travelers with headache. Lumbar puncture was not done in two cases: one patient was not admitted and the other had contraindication (congenital hydrocephalus).

Cerebrospinal fluid (CSF) examination showed an increased lymphocytosis in 5/6, suggesting a viral cause. All CSFs resulted positive for enterovirus (real time-polymerase chain reaction [RT-PCR] on the 5'UTR region of viral genome). This data was confirmed by isolation of an echovirus in 4/6 stool swabs (66%) and in 1/6 throat swab (16%). The virus was also isolated from the stools of the hydrocephalic patient.

The discrepancy between the number of enterovirus CSF-positive patients (6/6) and enterovirus stool-positive ones (4/6) is likely due to a much higher sensitivity of PCR technique compared with viral isolation in cell culture.

Enterovirus detection on rectal and pharyngeal swabs was done according to the WHO recommended protocols, by 37°C incubation on MRC-5, BGM, Hep2 and Vero cell lines, and examined for cytopathic effect daily for 21 days. Species identification

**Table 1** Clinical data of the symptomatic travelers

| Cases no | Age (years) and gender | Symptoms                         | Stiff neck | Echo-4 serology September 7, 2006 | Echo-4 serology September 28, 2006 | Throat swab echo isolation | Stool swab echo isolation | CSF PCR enterovirus | CSF cells | CSF Protocols | CSF glicor |
|----------|------------------------|----------------------------------|------------|-----------------------------------|------------------------------------|----------------------------|---------------------------|---------------------|-----------|---------------|------------|
| 1        | 24 f                   | Headache<br>Fever*               | No         | <1/8                              | 1/45                               | Neg                        | Pos                       | Pos                 | 1390      | 74            | 50         |
| 2        | 31 f                   | Headache<br>Fever                | No         | <1/8                              | 1/38                               | Neg                        | Pos                       | Pos                 | 260       | 43            | 59         |
| 3        | 18 f                   | Headache<br>Fever<br>Sore throat | Yes        | <1/8                              | 1/196                              | Pos                        | Neg                       | Pos                 | 650       | 76            | 67         |
| 4        | 18 f                   | Headache<br>Fever                | Yes        | <1/8                              | 1/275                              | Neg                        | Pos                       | Pos                 | 300       | 138           | 57         |
| 5        | 33 m                   | Headache<br>Fever<br>Vomiting    | No         | <1/8                              | 1/11                               | Neg                        | Neg                       | Pos                 | <4        | 41            | 65         |
| 6        | 21 m                   | Headache<br>Fever                | No         | <1/8                              | 1/78                               | Neg                        | Pos                       | —                   | —         | —             | —          |
| 7        | 18 f                   | Headache<br>Fever                | No         | <1/8                              | 1/316                              | Neg                        | Pos                       | Pos                 | 120       | 47            | 65         |
| 8        | 18 m                   | Headache<br>Fever                | No         | <1/8                              | 1/22                               | —                          | —                         | —                   | —         | —             | —          |

\*Axillary temperature >37.5°C.

was carried out by indirect fluorescent assays with monoclonal antibodies anti-enterovirus (Dako Cytomation, Glostrup, Denmark), anti-coxsackievirus, poliovirus, and echovirus (Chemicon International Inc., Temecula, CA, USA). Echovirus serotyping was done by seroneutralization of cytopathic effect by Lim and Benyesh-Melnick pools. Viral genome was detected by nested RT-PCR, after nucleic acid extraction and precipitation (Nested Enterovirus and Extragen, Amplimedical, Milan, Italy), with a test sensitivity of 200 copies/mL.

Serological tests performed, challenging patient serum with the isolated echovirus-4 in all 17 travelers, resulted negative at baseline in all cases but one (an asymptomatic girl). When they were repeated 3 weeks later, all the symptomatic and one of the asymptomatic travelers showed seroconversion.

Chest X-ray, cranial TC, and standard laboratory findings were all within normal limits. All patients recovered and no sequelae were recorded. The duration of the symptoms as well as of hospitalization ranged from 3 to 5 days for all patients.

All of them, including those who did not develop symptoms, had drunk tap water in a hostel 1 day before returning to Italy, ie, 2 to 3 days before the symptoms onset, and this was probably the only risk factor for enterovirus infections, compatible with the incubation period.

## Discussion and Conclusions

Every year about 80 million people travel from industrialized countries to developing regions.<sup>8</sup> Wilson et al. reported that a substantial proportion (22%)

of returned travelers with fever have an unspecified febrile episode.<sup>3</sup> In studies of patients in a tertiary care hospital, unidentified febrile syndrome accounted for 21% of cases,<sup>9</sup> 25% of cases among in-patients were not diagnosed,<sup>10</sup> and “viral illness” accounted for 34% of cases among children.<sup>11</sup>

Steffen et al. states that health problems (related or unrelated to travel) are reported by 22% to 64% of travelers to the developing world: most of these diseases are mild and self-limited, such as diarrhoea, as the most frequent illness occurring in 13.6% to 54.6% of travelers depends on travel conditions and destinations.<sup>12</sup>

Many of these cases remain undiagnosed due either to lack of laboratory facilities or to self-limiting short-duration diseases. As our report shows, enteroviruses may play a role in undiagnosed fevers in travelers. Clinical suspicion and the availability of adequate diagnostic facilities may limit the number of undiagnosed cases.

The transmission of enteroviruses is abetted by poor sanitary conditions and may occur via numerous routes including contaminated water, food, and fomites. In this cluster of cases, all patients were probably infected from the same source, because they became ill at the same time and no secondary cases (family or health personnel) were reported. Under these circumstances the cause seems to have been the contaminated tap water they drank in the hostel the day before returning to Italy; but in spite of this suspicion, the cause of the outbreak was not completely confirmed and remains speculative, although the clustering of the dates of onset (all from 48 to 72 h after return) clearly suggest a common source of exposure.

This is the first report about imported echovirus cluster in Italy: it may be assumed that usually the aseptic meningitis appears, due to its short incubation period, in the same country of acquired infection. The high attack rate is surprising (almost 50%, all with meningeal symptoms): this may be related to a particular virulence of this echovirus strain or, more probably, to the absence of immunity in all but one subject against echovirus-4. This serotype is one of the most often isolated in India, generally in children, whereas in Italy it is not particularly common. It has been suggested that accumulation of a “critical mass” of susceptible young children may be necessary to sustain epidemic transmission.<sup>13</sup> An outbreak with the same serotype was reported in Modena (Italy) in 2001: it was not imported and 23 of 25 patients were adults, confirming the low circulation and low immunity rate of this serotype in our country.<sup>14</sup>

Of all travelers, 80% did not follow the traditionally recommended dietary restrictions:<sup>1</sup> the risk for most travel-related diseases can be significantly reduced by applying preventive measures such as avoiding dangerous food items such as tap water, dairy products, ice-cream, salad, and seafood.

This is particularly important for travelers to India where the risk of becoming ill compared to other typical destinations is higher and not following traditionally recommended dietary restrictions in that country results in a twofold increased risk of illness.<sup>1</sup> This advice is especially important for young travelers who often travel under basic conditions and for elderly people, as the clinical consequences of diseases like enteroviral meningitis can be more severe for them.

### Acknowledgment

Thanks to Dr. Giorgio Pistono of virology laboratory department, Ospedale Amedeo di Savoia, Turin, Italy.

### Declaration of Interests

The authors state they have no conflicts of interest to declare.

### References

1. Rack J, Wichmann O, Kamara B, et al. Risk and spectrum of diseases in travellers to popular tourist destinations. *J Travel Med* 2005; 12: 248–253.
2. Bruni M, Steffen R. Impact of travel-related health impairments. *J Travel Med* 1997; 4: 61–64.
3. Wilson ME, Weld LH, Boggild A, et al. Fever in returned travellers: results from the GeoSentinel Surveillance Network. *Clin Infect Dis* 2007; 44: 1560–1568.
4. Freedman DO, Weld LH, Kozarsky PE, et al. Spectrum of disease and relation to place of exposure among ill returned travellers. *N Engl J Med* 2006; 354: 119–130.
5. Thoelen I, Lemey P, Van Der Donck I, et al. Molecular typing and epidemiology of enteroviruses identified from an outbreak of aseptic meningitis in Belgium during the summer of 2000. *J Med Virol* 2003; 70: 420–429.
6. Roth B, Enders M, Arents A, et al. Epidemiologic aspects and laboratory features of enterovirus infections in Western Germany, 2000–2005. *J Med Virol* 2007; 79: 956–962.
7. Antona D, Lévêque N, Chomel JJ, et al. Surveillance of enteroviruses in France, 2000–2004. *Eur J Clin Microbiol Infect Dis* 2007; 26: 403–412.
8. Steffen R, Tornieporth N, Clemens SA, et al. Epidemiology of travellers' diarrhoea: details of a global survey. *J Travel Med* 2004; 11: 231–237.
9. Stienlauf S, Segal G, Sidi Y, Schwartz E. Epidemiology of travel-related hospitalisation. *J Travel Med* 2005; 12: 136–141.
10. Doherty JF, Grant AD, Bryceson AD. Fever as the presenting complaint of travellers returning from the tropics. *QJM* 1995; 88: 277–281.
11. West NS, Riordan FA. Fever in returned travellers: a prospective review of hospital admissions for 2 (1/2) year period. *Arch Dis Child* 2003; 88: 432–434.
12. Steffen R, De Bernardis C, Baños A. Travel epidemiology—a global perspective. *Int J Antimicrob Agents* 2003; 21: 89–95.
13. Gondo K, Kusuhara K, Take H, Ueda K. Echovirus type 9 epidemic in Kagoshima, southern Japan: seroepidemiology and clinical observation of aseptic meningitis. *Pediatr Infect Dis J* 1995; 14: 787.
14. Portolani M, Pecorari M, Pietrosevoli P, et al. Outbreak of aseptic meningitis by echo 4: prevalence of clinical cases among adults. *New Microbiol* 2001; 24: 11–15.