Eur J Clin Microbiol Infect Dis (2007) 26:403–412
DOI 10.1007/s10096-007-0306-4

ARTICLE

Surveillance of enteroviruses in France, 2000–2004

D. Antona · N. Lévêque · J. J. Chomel · S. Dubrou · D. Lévy-Bruhl · B. Lina

Published online: 30 May 2007

Abstract In the context of poliomyelitis eradication, a reinforced sentinel laboratory network for surveillance of enteroviruses (RSE) was implemented in France in January 2000, and the purpose of this report is to describe the results of the five first years of surveillance. From 2000 to 2004, the RSE laboratory network performed detailed surveillance of the circulating enteroviruses. No wild-type poliovirus was isolated from humans during the 5 years of surveillance, although two imported vaccine polioviruses were detected. During the same period, Sabin-like polioviruses were identified on five occasions in the sludge from sewage treatment plants, but no wild-type poliovirus was found. Over the 5 years of surveillance, information was collected from 192,598 clinical samples, including 39,276 cerebrospinal fluid specimens, of which 14.7% were positive for enteroviruses, 45,889 stool samples (4.3% positive for enteroviruses), 70,330 throat swabs (2.2% positive) and 14,243 sera (1.4% positive). The ten main nonpolio enteroviruses typed were as follows, in decreasing order of frequency: E-30, E-13, E-6, CV-B5, E-11, CV-B4, E-9, E-7, CV-B1, and CV-B2. During the year 2000, an outbreak of aseptic meningitis due to three main enteroviruses (echoviruses type 30, 13, and 6) was monitored. Continued surveillance of enteroviruses is important to alert physicians and public health officials to changes in disease trends. Although the geographical coverage of the RSE network as well as the percentage of enteroviruses identified must be improved, the large number of samples tested for enteroviruses shows the ability of virology laboratories to detect the circulation of enteroviruses and to report the possible identification of poliovirus (wild-type, vaccine-derived, or Sabin-like).

Keywords Enterovirus · Poliovirus · Surveillance · France

D. Antona (✉) · D. Lévy-Bruhl
Département des Maladies Infectieuses,
Institut de Veille Sanitaire,
12 rue du Val d'Osne,
94415 Saint Maurice Cedex, France
e-mail: d.antona@invs.sante.fr

N. Lévêque · J. J. Chomel · B. Lina
Centre National de Référence pour les Entérovirus,
Centre Collaborateur OMS,
Laboratoire de Virologie du CHU de Lyon,
Domaine Rockefeller,
69373 Lyon Cedex 08, France

S. Dubrou
Laboratoire d'Hygiène de la Ville de Paris,
Mairie de Paris—DASES,
11 rue Georges Eastman,
75013 Paris, France

Introduction

The human enteroviruses (classified in four species, HEV-A to HEV-D) belong to the genus *Enterovirus*, family *Picornaviridae*. They include coxsackieviruses A and B, echoviruses, numbered enteroviruses, and polioviruses 1–3. By far, the most common form of infection by any of the enteroviruses is asymptomatic or is manifest by no more than minor malaise. This is true not only for poliovirus infections but also for infections by coxsackieviruses, echoviruses, and the newer enterovirus serotypes [1–3]. Except poliomyelitis, diseases associated with enterovirus infections are not notifiable in France.

The World Health Organisation (WHO) European region was declared poliovirus free in June 2002. In France, the last indigenous and imported cases of poliomyelitis occurred in 1989 and in 1995, respectively.

Although France did not implement acute flaccid paralysis (AFP) surveillance, the French national plan of action against poliomyelitis, endorsed in 1998, includes: (a) notification of polio cases (suspected and confirmed), as well as notification of wild-type, vaccine-derived polioviruses (VDPVs) and Sabin-like polioviruses identified in laboratory specimens, (b) overall immunisation with inactivated polio vaccine (the sole vaccine used in the country since 1986, with a 98% immunisation coverage in infants), and (c) surveillance of enteroviruses in humans and in environmental settings [4]. Furthermore, in 2004 France began implementing the process of laboratory containment of polioviruses, including not only wild-type strains but also VDPVs and Sabin-like polioviruses [5].

In the global context of poliomyelitis eradication, the surveillance of enteroviruses in humans was reinforced in 2000 [4, 6], and this paper describes the results of the first 5 years of this surveillance, performed as part of the national plan of action.

Materials and methods

Enhancement of enterovirus surveillance in humans

In January 2000, a reinforced sentinel laboratory network for surveillance of enteroviruses called RSE (Réseau de Surveillance des Entérovirus) was implemented as a result of the merging of two pre-existing surveillance networks. It is coordinated by the National Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS), which performs the epidemiological surveillance, together with the National Reference Centre for Enteroviruses (NRC), which performs microbiological investigations. The detection of any enterovirus from human samples is reported voluntarily to the RSE on a monthly basis by 30 laboratories scattered throughout mainland France (Fig. 1). All but one are located in a university setting and serve as regional reference laboratories, thereby collecting samples from at least 135 hospitals.

The objectives of the RSE network are to determine the patterns of circulation of the different enterovirus serotypes within the human population, to identify outbreaks (especially aseptic meningitis epidemics), to supply poliovirus surveillance by identifying imported strains (wild-type as well as vaccine and vaccine-derived strains), and to control any possible circulation of these viruses. The information collected includes the number and type of human specimens submitted for enterovirus detection by month, the hospital and ward of origin, and the distribution of specimens according to the age of the patients. For each sample positive for enterovirus, required information includes the date of collection, the clinical symptoms, the patient's age and gender, the methods used for virus detection (essentially culture and/or PCR), and, if available, the type of enterovirus identified by neutralisation or sequencing. In the RSE, the methods most commonly used for virus detection include virus isolation using at least one of the following cell lines: buffalo green monkey (BGM), human epidermoid carcinoma cells (Hep2), lung embryonic fibroblasts (MRC5), a recombinant murine cell line (L20B) which is specific for poliovirus isolation, and human rectal tumor cells (HRT), which are very sensitive for human parechoviruses. In most cases, specimens originate from stools, throat/nose or rectal swabs, and neutralization of the cytopathic effect is demonstrated in cell cultures using either monoclonal antiserum against the VP1 protein gene or Lim and Benyesh-Melnick (LBM) intersecting pools [7]. The reverse transcriptase polymerase chain reaction (RT-PCR) is used mainly on cerebrospinal fluid (CSF) and serum samples, with detection of the 5′ noncoding region of the genome, but this region is inappropriate for identification [8]. Consecutive identification can then be processed by amplifying and sequencing a region of the VP1 protein gene that encompasses the BC loop [9]; although few laboratories of the RSE actually do the sequencing, they may send the strains to the NRC.

A coordinated follow-up is done by both the InVS and the NRC and includes a monthly validation of routine activity data and regular feedback through interim and annual reports. In case of poliovirus detection, immediate reporting is required in order to conduct an investigation. The strain is sent to the NRC in Lyon for virus serotyping and intratyping differentiation. The result must be confirmed as soon as possible by the European WHO Regional Reference Laboratory for Poliomyelitis (Robert Koch Institute, Berlin, Germany).

Enterovirus surveillance in the environment

Following WHO recommendations, the National Commission for the Certification of Poliomyelitis Eradication included environmental surveillance of poliovirus in its 1998 plan of action [4]. In France, the Laboratory of Hygiene of Paris (LHVP) is conducting this surveillance since 1975 in the "Ile de France" region, an urban area including Paris and environs, and in which 8 million people are living (13% of the total population). Each year, around 48 activated sludge samples and 72 sewage samples are collected from four wastewater treatment plants according to a 1-L grab sample procedure. Samples are obtained monthly and treated for viral examination without any concentration step. Four 96-well culture plates of BGM and Hep-2 cells seeded in suspension are inoculated with a 5-ml sample. Two blind passages are processed. Viral isolates are selected according to their typical cytopathic effect and

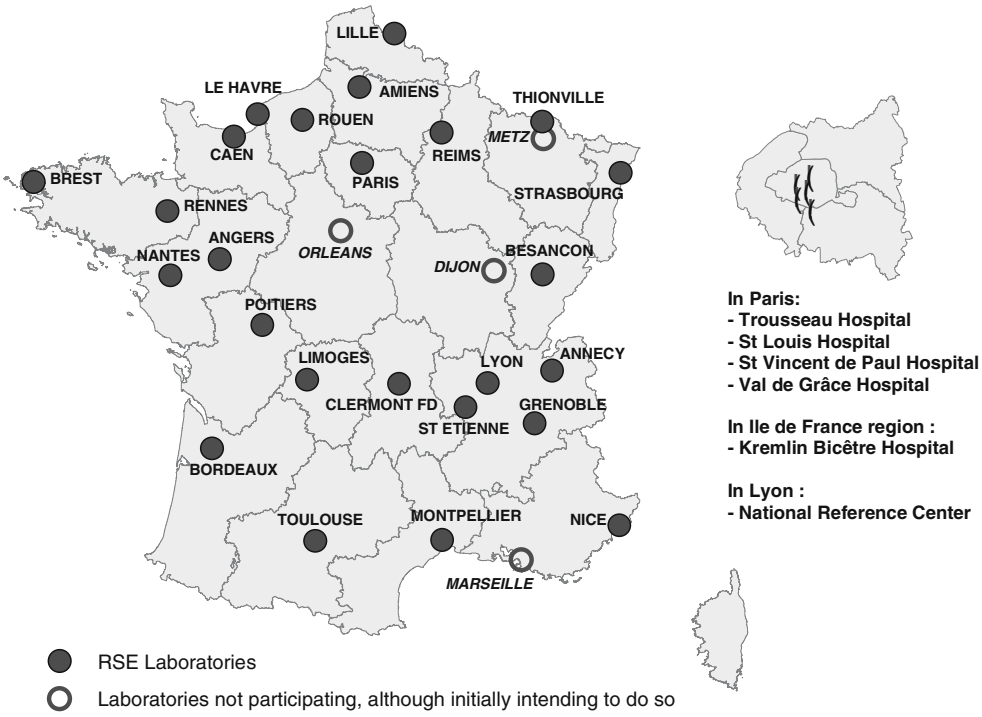

Fig. 1 Enhanced enterovirus surveillance: distribution of RSE laboratories as of 01/01/2005, France

tested for poliovirus identification by neutralization tests as described above. All poliovirus strains as well as a portion of other viral strains are sent to the NRC for virus identification and serotyping. In case of poliovirus identification, typing and intratyping differentiation is performed by the NRC and the result confirmed by the European WHO Regional Reference Laboratory for Poliomyelitis in Berlin.

Results

Population surveillance

From 2000 to 2004, neither a confirmed nor a suspected case of poliomyelitis was reported, and neither wild-type nor VDPV polioviruses were identified from the human biological samples submitted for virus serotyping within or outside the RSE network.

Overall data

The RSE network collected information from 192,598 samples including 39,276 (20.4%) CSF samples (of which 14.7% positive for enteroviruses), 45,889 (23.8%) stool samples (4.3% positive), 70,330 (36.5%) throat swabs (2.2% positive), and 14,243 (7.4%) serum samples (1.4% positive). Table 1 shows their distribution by type of sample and age group, along with the percentage of positive samples in each subgroup. The highest percentage of positive detections was found in CSF specimens from children aged 5–14 years, of which 35.6% tested positive. Data collection increased over time, with 41,482 samples tested in 2004, versus 36,832 in 2000. As shown on Fig. 2, the geographic coverage of the network has improved over time, including data from southeastern France since the beginning of 2004.

Poliovirus

Children under 15 (WHO target population for AFP surveillance) accounted for 85% of the 34,247 stool samples tested for which the age of the patient was specified. During the 5 years of surveillance, two Sabin-like polioviruses were identified among a total of 45,889 stool specimens tested. The first one was a type 3 Sabin poliovirus found in 2002 in the diarrheic stools of a 4-month-old boy. This child had come from Algeria, where he had received two doses of oral polio vaccine (OPV). The second poliovirus was a type 1 strain found in 2003 in an 8-day-old baby born in Morocco, another country where OPV is used. Neither strain was isolated in the clinical context of paralysis; both were obtained from stool specimens tested as part of a check-up.

Nonpolio enteroviruses

Of the 192,598 samples analyzed by the RSE laboratories, enteroviruses were detected in 9,603 (5%), obtained from 8,978 patients. The circulation of enteroviruses showed

Table 1 Specimens submitted for detection of enterovirus and proportions with positive results: distribution of 192,598 specimens by origin of specimen and patient age group (RSE data, France, 2000–2004)

Type of specimen	No. of specimens tested (no. positive for enteroviruses)							
	Age < 1 year	Age 1–4 years	Age 5–14 years	Age 15–24 years	Age 25–49 years	Age ≥ 50 years	Age not specified[a]	Total
Cerebrospinal fluid	6,324 (17.1%)	2,992 (19.0%)	3,675 (35.6%)	2,086 (35.6%)	5,980 (10.1%)	7,095 (1.9%)	11,124 (15.6%)	39,276 (14.7%)
Stool	14,796 (5.4%)	9,515 (4.5%)	4,735 (4.6%)	1,345 (5.0%)	1,754 (10.0%)	2,102 (1.2%)	11,642 (2.5%)	45,889 (4.3%)
Nose, throat	21,406 (2.3%)	13,635 (2.7%)	6,669 (3.9%)	2,972 (2.2%)	7,457 (1.3%)	9,368 (0.3%)	8,823 (2.0%)	70,330 (2.2%)
Serum	773 (7.5%)	1,087 (1.7%)	1,149 (1.7%)	1,392 (0.7%)	4,488 (1.0%)	4,420 (0.5%)	934 (3.5%)	14,243 (1.4%)
Other (urine, mucous membranes, skin, amniotic fluid, biopsies)	3,495 (0.9%)	938 (3.0%)	1,225 (0.8%)	1,863 (0.4%)	6,604 (0.4%)	5,197 (0.5%)	3,538 (0.4%)	22,860 (0.6%)

[a] Age not specified: a few laboratories do not specify the age for all patients tested, and the age is known only when patients are tested positive. Therefore, the latest were excluded from the computation of percentages of positive cases by age groups.

roughly the same seasonal pattern throughout the observation period, except in 2000, when an outbreak of aseptic meningitis occurred. As expected in a temperate country [1–3], the number of cases increased each year during summer and the beginning of fall, with a peak in July (uniformly observed in week 27), and then slowly decreased, except in 2004, when a second peak of the same size was observed during week 43 (Fig. 3).

The age distribution of patients who tested positive (age known for 8,914 patients) was as follows: 3,024 (34%) patients under 1 year of age, 1,678 (19%) between 1 and 4 years of age, 2,304 (26%) between 5 and 14 years of age, 456 (5%) between 15 and 24 years of age, 1,179 (13%) between 25 and 49 years of age, and 273 (3%) more than 50 years of age. The case distribution according to gender shows a majority of male patients, with a sex ratio of 1.5 (5,018 males/3,436 females).

The RSE surveillance system is based on a voluntary laboratory network and is therefore not exhaustive in terms of the clinical description of cases. A precise clinical context was available for 5,445 (61%) patients. Central nervous system diseases were the most prevalent (n=3,752; 68.9%), among which meningitis counted for 67% (n=3,646) and encephalitis for 0.7% (n=37). Although the network is not intended to detect AFP cases, five cases of Guillain-Barré syndrome, one case of transverse myelitis,

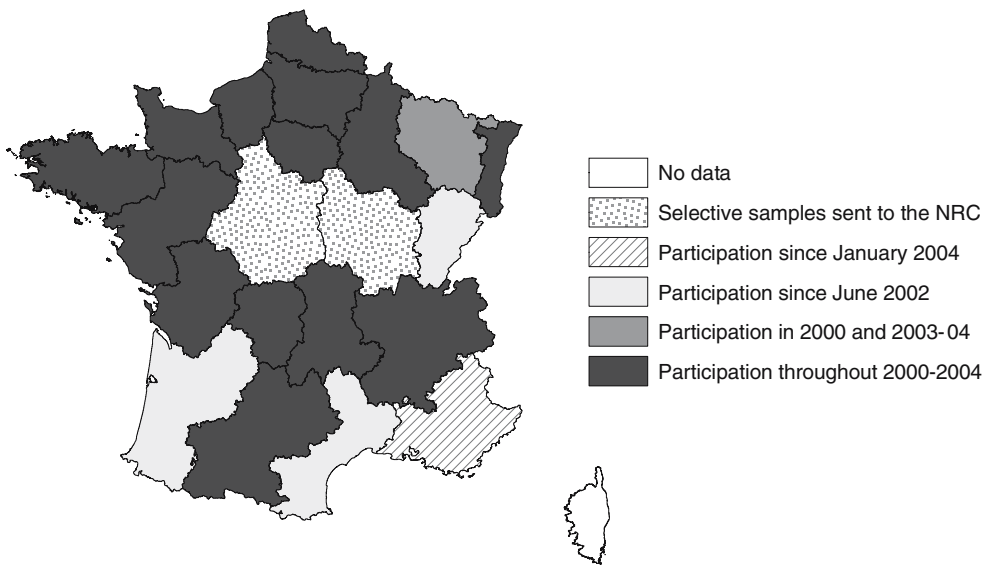

Fig. 2 Enhanced enterovirus surveillance: evolution of RSE geographic coverage, France 2000–2004

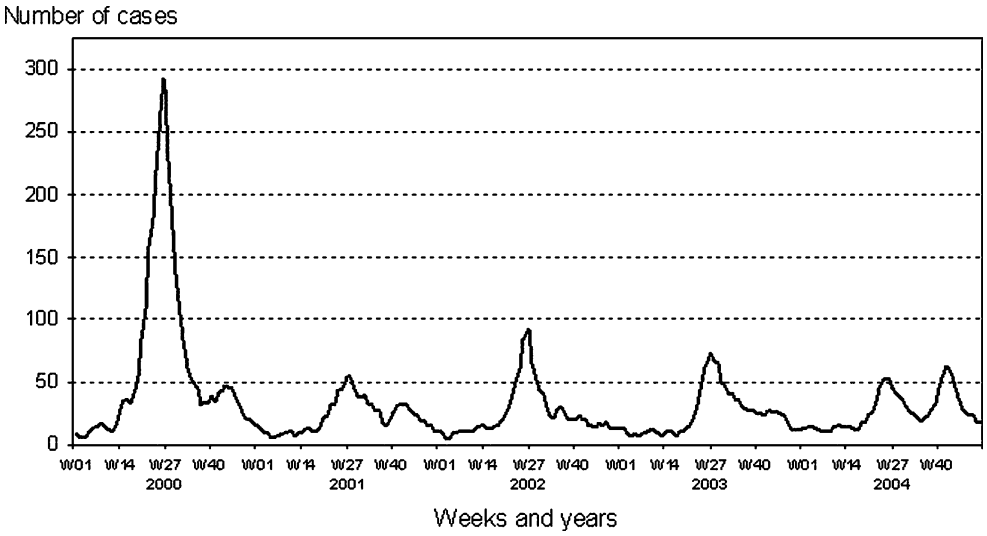

Fig. 3 Seasonal pattern of enterovirus circulation, RSE data, France 2000–2004 (distribution of cases per week, moving averages)

and four cases of mild paresis were described in children below 15 years of age. For each case, in addition to CSF samples, stools samples or rectal swabs and/or throat swabs were obtained, and the enteroviruses identified were CV-B2, CV-B3, and E-6.

The main other clinical syndromes comprised gastrointestinal, neuromuscular, and respiratory diseases, along with a few cases of cardiac diseases and hand, foot, and mouth disease. Table 2 shows the distribution of clinical syndromes according to age group.

A total of 8,978 patients tested positive for enteroviruses, but the strain was not subsequently typed in 4,758 (53%) patients and was typed as nonpolio enterovirus in 1,463 (16.3%) patients. Definitive identification was achieved in 2,757 (30.7%) patients (Table 3).

The ten main serotypes of nonpolio enterovirus circulating in humans from 2000 to 2004 belonged to the species Human Enterovirus B (HEV-B) and were, in decreasing order, echovirus 30 (E-30), E-13, E-6, coxsackievirus B5 (CV-B5), E-11, CV-B4, E-9, E-7, CV-B1, and CV-B2.

During the 2000 meningitis outbreak, three main serotypes were isolated, accounting for 83% of the enteroviruses identified: E-30 (40%), E-13 (32%), and E-6 (11%) [10]. The epidemic started in the west of France (Brittany), spread to the Loire Valley, with a majority of cases caused by E-30, and then to the Picardie and Paris regions, where E-13 was mainly involved. E-6 circulated at a lower level through all regions at the same time, and continued to do so during 2001 and 2002, while E-30 and E-13 circulated less or not at all during 2001–2004. In 2002, the main serotype circulating was E-11, which spread throughout the country all year long, without any specific outbreak in time or place. In 2004, the main type circulating in summer was CV-B4 and in fall E-30, while E-7 and CV-B2 were circulating at a lower level during the entire period.

Table 2 Distribution of clinical syndromes reported for 5,404 patients positive for nonpolio enteroviruses, according to patient age group (RSE data, France 2000–2004)

Clinical syndrome	No. of positive patients						Total
	Age < 1 year	Age 1–4 years	Age 5–14 years	Age 15–24 years	Age 25–49 years	Age ≥ 50 years	
Central nervous system	757	637	1,329	254	613	124	3,714 (68.7%)
Infectious (no further details reported)	501	140	58	10	30	8	747 (13.8%)
Gastrointestinal	171	143	51	7	7	7	386 (7.2%)
Respiratory	126	69	15	6	4	7	227 (4.2%)
Neuromuscular	27	17	35	4	19	7	109 (2.0%)
Cardiac	7	6	3	2	14	13	45 (0.8%)
Hand, foot & mouth	7	22	2	1	3	2	37 (0.7%)
Other	44	35	20	8	18	14	139 (2.6%)
Total	1,640 (30.3%)	1,069 (19.8%)	1,513 (28.0%)	292 (5.4%)	708 (13.1%)	182 (3.4%)	5,404 (100%)

Table 3 Results of serotyping of nonpolio enteroviruses from 2,757 patients (RSE, France, 2000–2004)

Serotype	2000	2001	2002	2003	2004	Total
E-30	*525*	*44*	15	6	*49*	*639* (*23.2%*)
E-13	*425*	*13*	4	3	0	*445* (*16.1%*)
E-6	*147*	*62*	*74*	*10*	*10*	*303* (*11.0%*)
CV-B5	*84*	4	*18*	*59*	*19*	*184* (*6.7%*)
E-11	*12*	*18*	*115*	*15*	*10*	*170* (*6.2%*)
CV-B4	*38*	*23*	5	*15*	*34*	*115* (*4.2%*)
E-9	1	*23*	*16*	*40*	*23*	*103* (*3.7%*)
E-7	8	4	0	*23*	*54*	*89* (*3.2%*)
CV-B1	*11*	1	*34*	*30*	5	*81* (*2.9%*)
CV-B2	*14*	*20*	0	6	*41*	*81* (*2.9%*)
CV-B3	0	3	*25*	*21*	*16*	65 (2.4%)
E-5	*8*	4	*40*	6	6	64 (2.3%)
E-4	0	2	*23*	*18*	3	46 (1.7%)
CV-A9	3	*8*	*17*	5	8	41 (1.5%)
E-18	*11*	5	*17*	2	2	37 (1.3%)
E-25	0	1	4	*10*	*19*	34 (1.2%)
E-16	3	*9*	8	6	4	30 (1.1%)
E-21	6	3	3	6	5	23 (0.8%)
E-3	3	1	10	2	3	19 (0.7%)
E-17	*8*	3	1	3	4	19 (0.7%)
HPEV 1[a]	1	1	12	2	0	16 (0.6%)
E-20	0	2	4	4	1	11 (0.4%)
E-14	3	*7*	1	0	0	11 (0.4%)
CV-A24	0	2	4	4	0	10 (0.4%)
CV-A16	1	2	2	2	3	10 (0.4%)
E-31	0	3	2	1	3	9 (0.3%)
EV-71	1	0	1	5	1	8 (0.3%)
Other[b]	10	19	9	28	28	94
Total	1,323	287	464	332	351	2,757 (100%)

All strains identified belonged to the human enterovirus B species (HEV-B), except CV-A16 and CV-A24 (HEV-C) and EV-71 (HEV-A).
Italics indicate the ten most common serotypes found each year and in total
E echovirus, *CV-A* or *CV-B* coxsackievirus A or B, *EV* enterovirus
[a] Parechovirus 1 (E-22 previously) is now classified among *Parechovirus* and no longer among *Enterovirus*
[b] Other serotypes identified, each of which accounted for less than 0.3% of the 5-year total

The distribution of the ten main serotypes is related to the age of the patients (Table 4). As a whole, they were more frequently identified among children below 15 years of age, with E-30 found more specifically among children between 5 and 14 years, and E-11, CV-B4, E-9, E-7, and CV-B2 in infants below 12 months.

Table 5 shows the distribution of the ten main serotypes according to clinical syndrome. As expected, the most frequent syndrome involved the central nervous system for most of the serotypes.

Environmental surveillance

From 2000 to 2004, 234 of the 237 (99%) sludge samples and 277 of the 356 (78%) sewage wastewater samples were positive for viruses. Of the viral strains isolated from the environment, 5,379 of 9,833 (55%) from sludge samples and 2,299 of 3,230 (71%) from sewage specimens were tested for poliovirus through neutralization of the cytopathic effect.

The NRC received 1,015 isolates for typing. Coxsackieviruses B accounted for 62% of the strains, coxsackieviruses A for 0.5%, echoviruses for 3%, polioviruses for 0.5%, adenoviruses for 19%, and reoviruses for 15%.

Sabin-like polioviruses were identified on five occasions in the sludge from sewage treatment plants: serotype 2 in 2000, 2003, and 2004, and serotype 1 in 2001. No wild-type poliovirus was found.

The E-6 and E-13 types accounted for 3% of the strains identified during the summer of 2000, following the meningitis outbreak caused by these two types. As the aim of identification is based on polioviruses, it is difficult to draw precise correlations between the serotypes found in people living in the Paris region and those found in the

Table 4 Distribution of the ten main nonpolio enterovirus serotypes identified (n=2,154 patients), according to patients' age group (RSE data, France, 2000–2004)

Serotype	No. of patients tested positive for enteroviruses (% from each age group)						Total
	Age <1 year	Age 1–4 years	Age 5–14 years	Age 15–24 years	Age 25–49 years	Age ≥ 50 years	
E-30	53 (8.5%)	104 (16.6%)	260 (41.5%)	46 (7.3%)	154 (24.6%)	10 (1.6%)	627 (100%)
E-13	113 (28.1%)	65 (16.2%)	137 (34.1%)	31 (7.7%)	56 (13.9%)	–	402 (100%)
E-6	78 (25.7%)	95 (31.4%)	84 (27.7%)	13 (4.3%)	27 (8.9%)	6 (2.0%)	303 (100%)
CV-B5	68 (37.2%)	47 (25.7%)	33 (18.0%)	4 (2.2%)	20 (10.9%)	11 (6.0%)	183 (100%)
E-11	101 (59.4%)	41 (24.1%)	19 (11.2%)	1 (0.6%)	8 (4.7%)	–	170 (100%)
CV-B4	48 (41.7%)	26 (22.6%)	21 (18.3%)	1 (0.9%)	14 (12.2%)	5(4.3%)	115 (100%)
E-9	50 (48.5%)	26 (25.2%)	16 (15.5%)	5 (4.9%)	6 (5.8%)	–	103 (100%)
E-7	49 (55.1%)	28 (31.5%)	9 (10.1%)	–	2 (2.2%)	1 (1.1%)	89 (100%)
CV-B1	30 (37.0%)	29 (35.8%)	11 (13.6%)	1 (1.2%)	8 (9.9%)	2 (2.5%)	81 (100%)
CV-B2	40 (49.4%)	25 (30.9%)	9 (11.1%)	1 (1.2%)	4 (4.9%)	2 (2.5%)	81 (100%)
Total	630 (29.2%)	486 (22.6%)	599 (27.8%)	103 (4.8%)	299 (13.9%)	37 (1.7%)	2,154 (100%)

E echovirus, *CV-B* coxsackievirus B

environment, due to the small numbers of enterovirus strains isolated in environmental samples and submitted for serotyping.

Discussion

From 2000 to 2004, the RSE laboratory network performed detailed surveillance of the circulating enteroviruses. No wild-type poliovirus was isolated during the 5 years of surveillance, although two imported vaccine polioviruses were detected in humans.

The geographic coverage of the RSE network has improved over time, although information collected in the southeast of France is still incomplete. Because this region, which borders the Mediterranean Sea, may be the site of a possible reintroduction of polioviruses to France from endemic countries, the surveillance would benefit from broader participation in the RSE network.

Enteroviruses are found in all parts of the world. Widely distributed throughout the year in tropical and semitropical regions, they are present in temperate climates at low levels in winter and spring but are found more commonly in summer and fall. Consistent with these data, our surveillance system showed a seasonal pattern of distribution, with the circulation of enteroviruses peaking in summer and slowly decreasing in the early fall. During 2004, which was not an epidemic year, a second peak of circulation was observed in the fall. This peak was slightly higher than the summer one and was probably linked to persisting mild temperatures, although no further increases in cases occurred later during the winter, as observed in 1999–2000 [11].

Over those years of surveillance, the ten main nonpolio enteroviruses typed were as follows, in decreasing order: E-30, E-13, E-6, CV-B5, E-11, CV-B4, E-9, E-7, CV-B1, and CV-B2. During 2000, the RSE described an outbreak of aseptic meningitis due to three main enteroviruses: E-30, E-13, and E-6. E-6 and E-30 are known to cause outbreaks of aseptic meningitis [10]. During the same time period, reports from other European countries showed concurrent increases in the incidence of E-30 as well as outbreaks of E-13 infections. In Belgium, during the 2000 outbreak, predominant enteroviruses were the same as those observed in France: E-30 (31%), E-13 (23%), and E-6 (20%) [12]. Outbreaks of E-30 were reported from Iceland, Kosovo, and the Netherlands in 2000 and from England and Wales, Scotland, Germany, and Ireland in 2001 [13].

In 2000, E-13 caused outbreaks in Spain, England and Wales, Scotland, Ireland, Germany, and the Netherlands [13–15]. Prior to 2000, E-13 was one of the less commonly isolated echovirus types, and since 1976, not any E-13 related outbreak was observed through previous surveillance in France. In England and Wales, between 1990 and 1999, out of 4,405 echoviruses isolated and typed, 25 (0.02%) were found to be type 13 [13]. In Spain, prior to the 2000 outbreak, the main circulating enteroviruses were E-30, E-9, E-6, and E-4 [16]. In Germany in 2001, the number of identifications of E-13 was more than double that in the previous year. Another small outbreak occurred in the Netherlands in which this virus accounted for 31 of 473 (7%) enteroviruses isolated in 2001 [13, 15]. Outside of European temperate countries, in the USA, E-13 accounted for 376 of the 1,584 (24%) enterovirus isolates reported in 2001 (29% of the isolates reported had a known serotype), compared with 74 isolates reported during 1970–

Table 5 Distribution of the 10 main serotypes of nonpolio enteroviruses identified from 1,656 patients, according to clinical syndrome, RSE data, 2000–2004

Serotype	No. of patients tested positive for enteroviruses (% from each clinical syndrome)								Total
	CNS syndrome	Infectious	Gastrointestinal	Respiratory	Neuromuscular	Cardiac	Hand, foot & mouth	Other	
E-30	471 (90.8%)	13 (2.5%)	10 (1.8%)	5 (1.0%)	16 (3.1%)	–	1 (0.2%)	3 (0.6%)	519 (100%)
E-13	281 (81.9%)	23 (6.7%)	15 (4.4%)	8 (2.3%)	11 (3.2%)	1 (0.3%)	–	4 (1.2%)	343 (100%)
E-6	176 (71.3%)	26 (10.5%)	16 (6.5%)	11 (4.5%)	7 (2.8%)	2 (0.8%)	1 (0.4%)	8 (3.2%)	247 (100%)
E-11	37 (33.0%)	28 (25.0%)	17 (15.2%)	21 (18.8%)	3 (2.7%)	–	1 (0.9%)	5 (4.5%)	112 (100%)
CV-B5	57 (51.4%)	22 (19.8%)	8 (7.2%)	13 (11.7%)	4 (3.6%)	1 (0.9%)	–	6 (5.4%)	111 (100%)
E-9	40 (49.4%)	24 (29.6%)	9 (11.1%)	4 (4.9%)	–	–	–	4 (4.9%)	81 (100%)
E-7	27 (36.0%)	28 (37.3%)	5 (6.7%)	6 (8.0%)	–	–	–	9 (12.0%)	75 (100%)
CV-B1	18 (29.5%)	23 (37.6%)	10 (16.4%)	4 (6.6%)	4 (6.6%)	–	–	2 (3.3%)	61 (100%)
CV-B4	19 (32.8%)	8 (13.8%)	12 (20.7%)	12 (20.7%)	1 (1.7%)		1 (1.7%)	5 (8.6%)	58 (100%)
CV-B2	14 (28.6%)	11 (22.4%)	10 (20.4%)	10 (20.4%)	1 (2.0%)	–	–	3 (6.1%)	49 (100%)
Total	1,140	206	112	94	47	4	4	49	1,656

CNS central nervous system, *E* echovirus, *CV-B* coxsackievirus B

2000 [17, 18]. During the following years, predominant circulating serotypes were E-9 and E-30 [19]. All characterized E-13 isolates from the USA, Europe, Asia, and Oceania recovered in 2000–2002 were at least 95% identical to each other in VP1 capsid gene sequence, but they were genetically distinct from E-13 isolates recovered before 2000 [17].

As far as clinical aspects are concerned, our findings are consistent with the literature [1–3, 17–19], with a majority of cases observed in people below 15 years of age and the most prevalent syndromes being diseases of the central nervous system, mainly aseptic meningitis, linked to the ten main enteroviruses typed. In the literature, the most frequent serotypes known to be involved in aseptic meningitis outbreaks are CV-A7, CV-A9, CV-B1 to CV-B5, E-2 to E-4, E-7, E-9, E-11, E-14, E-16 to E-19, E-25, E-30, E-33, and enterovirus 71 (EV-71). Sporadic cases of encephalitis may be observed, too (an average of 10 cases per year recorded in France through the RSE), as well as paralysis and ataxias alone [1–3].

The other clinical features linked to enteroviruses include hand, foot, and mouth disease, due mainly to CV-A16 and EV-71, mild respiratory syndromes related to CV-A21 and CV-B2 to CV-B5, acute myocarditis and pericarditis involving CV-B3, acute hemorrhagic conjunctivitis epidemics caused by the EV-70 and the CV-A24 variant, and digestive syndromes linked to echoviruses [2, 20]. The main types linked to neonatal diseases are E-11 (half of published cases) and CV-B (except type 6), which are involved in one-third of the cases [1].

If a large outbreak of viral meningitis occurs, as in 2000, it may become problematic to conduct a prospective identification of viruses on a large scale. Moreover, differentiation between polioviruses and nonpolio enteroviruses was not possible through the PCR assay most commonly used in the laboratories of the RSE. Each year, numerous enterovirus strains remained untypeable by the conventional neutralisation assay using Melnick intersecting pools because these pools were prepared in the 1960s from old strains described at that time and which probably had since undergone genetic changes [1]. Moreover, since then, new serotypes were discovered and were not included in the LBM pools. Partial sequencing of the VP1 protein gene and sequences alignment in the international GenBank databank made it possible to type 50% of the strains previously classified as untypeable. Furthermore, novel enteroviruses such as EV-74, EV-75, EV-76, EV-77, and EV-78 have been characterised by genotyping [21–23].

This explains why, in the RSE surveillance, half of the specimens were only characterized as enterovirus positive. To deal with this problem, two strategies were suggested. The first involves the collection of stools, rectal swabs, or throat swabs in addition to CSF specimens from patients suffering from aseptic meningitis in order to perform enterovirus isolation and serotyping for epidemiological data. The second involves the use of a second, polio-specific PCR on each CSF specimen that tests positive for enteroviruses. Characterisation of the enterovirus species A–D can be performed directly from the sample by using specific primers. More recently, the NRC has evaluated a new enterovirus PCR assay that is as sensitive as the one currently in use, which would make direct genotyping from CSF specimens possible. It performs direct sequencing the 3′ part of the VP1 coding gene using defective primers. The limiting factor remains the low quantity of RNA found in CSF. Up to now, in France, the first strategy has been chosen as most suitable to field activities and financial constraints.

No wild-type poliovirus strain was detected from sludge or sewage during the 5 years of surveillance. Prior to 2000, the number of poliovirus isolates in the Parisian region decreased regularly since 1982 and corresponded to around one strain per year since 1991, in accordance with the decrease in the poliomyelitis incidence and the recommendation of exclusive use of inactivated polio vaccine in France since 1982. Up until 1990, poliovirus accounted for 10% of the enterovirus isolates recovered, with an equal distribution between wild-type, vaccine-derived, and indeterminate strains. Since 1990, only one wild-type poliovirus has been identified, in 1996. The strain belonged to the same lineage as the poliovirus 3-21267/Morocco 1977. Vaccine strains accounted for a very small part of the enteroviruses isolated. This environmental surveillance complements the surveillance of enteroviruses in humans in France, even though no direct correlation can be easily made between the two surveillance systems.

Continued surveillance of enteroviruses is important to alert physicians and public health officials of changes in disease trends. Since the establishment of the RSE in 2000, 30 laboratories have participated regularly in the surveillance network, and the high number of samples tested for enteroviruses shows the ability of the virology laboratories to monitor the circulation of enteroviruses and to report possible identifications of poliovirus (wild-type, Sabin-like, or VDPV). Monitoring the detection of poliovirus is an important aspect of the RSE's mission. Because of the immunization policy in place for the past 25 years, which requires the use of inactivated poliovirus vaccine only, the high immunization coverage rates, and the elimination of indigenous cases since 1989, reports of poliovirus to RSE reflect strains imported to France. Only two imported vaccine polioviruses were reported during the 5 years of surveillance. Since then, for the first time, one type 2 VDPV was isolated in February 2006 from the stool samples of an 11-month-old child, transferred from Tunis to Paris for a bone marrow transplant in the context of an immune deficit at birth. He had received oral polio vaccine in Tunisia. He presented with severe diarrhoea, but no neurological signs; the strain was confirmed as nonvirulent by both the NRC in Lyon and the RKI in Berlin.

Because the possibility exists of an importation of a virulent VDPV or wild-type poliovirus from an endemic zone, it is necessary to enhance the capacity of the RSE network. This requires improvement in the geographic coverage (especially in south eastern France) as well as in the number of polio/nonpolio characterizations of enterovirus-positive specimens.

Acknowledgements Thanks to the RSE participating teams in: Amiens (D. Hecquet, G Duverlie), Angers (S. Kouyoumdjian, F. Lunel-Fabiani), Annecy (B. Chanzy), Besançon (A. Coaquette, G. Herbein), Bordeaux (H. Fleury, M.E. Lafon), Brest (M.C. Legrand, B. Picard), Caen (J. Petitjean, F. Freymuth), Clermont Ferrand (C. Henquell, H. Peigue-Lafeuille), Grenoble (B. Gratacap, J.M. Seigneurin), Kremlin-Bicêtre (I. Bouillery, C. Pallier, P. Nordman), Le Havre (A. Morel), Lille (A. De Wilde, P. Wattre, D. Hober), Limoges (S. Alain, F. Denis, S. Rogez), Lyon (J.J. Chomel, N. Lévêque, B. Lina), Montpellier (M. Segondy, V. Foulongne), Nantes (S. Billaudel, M. Coste-Burel), Nice (A. Caramella, J.C. Lefebvre), Poitiers (A. Bourgoin, G. Agius), Reims (J. Carquin, L. Andreoletti, C. de Champs), Rennes (A. Depatureaux, R. Colimon), Rouen (M. Gueudin, F. Simon, C. Buffet-Janvresse), St. Etienne (O. Shabir, B. Pozzetto), Strasbourg (J.P. Gut, F. Stoll Keller), Thionville (C. Delamare, F. Hussenet), Toulouse (C. Mengelle, J. Izopet), Paris—St. Louis (C. Scieux, P. Lagrange), Paris—St. Vincent de Paul (P. Lebon), Paris—Trousseau (A. Dehée, A. Garbag-Chenon), and Paris—Val de Grâce (J. Maslin, E. Nicand, R. Teyssou). Thanks, also, to the team at the Laboratoire d'Hygiène de la Ville de Paris (S. Dubrou, D. Carlier), and special thanks to the technicians in each participating laboratory.

References

1. Melnick JL (1997) Poliovirus and other enteroviruses. In: Evans AS, Kaslow RA (eds) Viral infections of humans. Epidemiology and control. Plenum, New York, pp 583–663
2. Peigue-Lafeuille H (2003) Picornaviridae. In: Huraux JM, Nicolas JC, Agut H, Peigue-Lafeuille H (eds) Traité de virologie médicale. Editeur ESTEM, Paris, pp 399–405
3. Pallansch MA, Roos RP (2001) Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knippe DM, Howley PM (eds) Fields virology. Lippincott Williams and Wilkins, Philadelphia, pp 723–775
4. Anonymous (2000) Plan d'action de la Commission Nationale de Certification de l'éradication de la Poliomyélite: actualisation du plan d'action de juin 1998. Conduite à tenir devant un cas de polio suspect ou confirmé ou devant un isolement de poliovirus. http://www.invs.sante.fr/beh/2000/004647/beh_46_47_2000.pdf. Cited 13 Nov 2000
5. Lerasle S (2005) Confinement des poliovirus en laboratoire. http://www.invs.sante.fr/beh/2005/39_40/beh_39_40_2005.pdf. Cited 11 Oct 2005

6. Antona D (2002) L'éradication des maladies infectieuses: l'exemple de la poliomyélite. Médecine/Sciences 18:55–61
7. Lim KA, Benyesh-Melnick M (1960) Typing of viruses by combinations of antiserum pools. Application to typing of enteroviruses (coxsackie and ECHO). J Immunol 84:309–317
8. Lina B, Pozzetto B, Andreoletti L, Beguier E, Bourlet T, Dussaix E, Grangeot-Keros L, Gratacap-Cavallier B, Henquell C, Legrand-Quillien MC et al (1996) Multicenter evaluating of a commercially available PCR assay for diagnosing enterovirus infection in a panel of cerebrospinal fluid specimens. J Clin Microbiol 34:3002–3006
9. Oberste MS, Nix WA, Maher K, Pallansch MA (2003) Improved molecular identification of enteroviruses by RT-PCR and amplicon sequencing. J Clin Virol 26:375–377
10. Chomel JJ, Antona D, Thouvenot D, Lina B (2003) Three ECHOvirus serotypes responsible for outbreak of aseptic meningitis in Rhone-Alpes region, France. Eur J Clin Microbiol Infect Dis 22:191–193
11. Chambon M, Archimbaud C, Bailly JL, Henquell C, Regagnon C, Charbonne F, Peigue-Lafeuille H (2001) Circulation of enteroviruses and persistence of meningitis cases in the winter of 1999–2000. J Med Virol 2:340–347
12. Thoelen I, Lemey P, Van Der Donck I, Beuselinck K, Lindberg AM, Van Ranst M (2003) Molecular typing and epidemiology of enteroviruses identified from an outbreak of aseptic meningitis in Belgium during the summer of 2000. J Med Virol 70:420–429
13. Noah N, Reid F (2002) Recent increases in incidence of echoviruses 13 and 30 around Europe. http://www.eurosurveillance.org/ew/2002/020214.asp#4. Cited 14 Feb 2002
14. Trallero G, Casas I, Avellon A, Perez C, Tenorio A, De La Loma A (2003) First epidemic of aseptic meningitis due to echovirus type 13 among Spanish children. Epidemiol Infect 130:251–256
15. Diedrich S, Schreier E (2001) Aseptic meningitis in Germany associated with echovirus type 13. BMC Infect Dis 1:14
16. Trallero G, Casas I, Tenorio A, Echevarria JE, Castellanos A, Lozano A, Brena PP (2000) Enteroviruses in Spain: virological and epidemiological studies over 10 years (1988–97). Epidemiol Infect 124:497–506
17. Mullins JA, Khetsuriani N, Nix WA, Oberste MS, LaMonte A, Kilpatrick DR, Dunn J, Langer J, McMinn P, Huang QS, Grimwood K, Huang C, Pallansch MA (2004) Emergence of echovirus type 13 as a prominent enterovirus. Clin Infect Dis 38:70–77
18. Centers for Disease Control and Prevention (2002) Enterovirus surveillance—United States, 2000–2001. Morb Mort Wkly Rep 5146:1047–1049
19. Centers for Disease Control and Prevention (2006) Enterovirus surveillance—United States, 2002–2004. Morb Mort Wkly Rep 5506:153–156
20. Dussart P, Cartet G, Huguet P, Lévêque N, Hajjar C, Morvan J, Vanderkerckhove J, Ferret K, Lina B, Chomel JJ, Norder N (2005) An outbreak of acute hemorrhagic conjunctivitis during 2003 in French Guiana and Guadeloupe caused by coxsackie A24 variant: phylogenetic analysis reveals Asian import. J Med Virol 75:559–565
21. Oberste MS, Michele SM, Maher K, Schnurr D, Cisterna D, Junttila N, Uddin M, Chomel JJ, Lau CS, Ridha W, Al-Busaidy S, Norder H, Magnius LO, Pallansch MA (2004) Molecular identification and characterization of two proposed new enterovirus serotypes, EV74 and EV75. J Gen Virol 85:3205–3212
22. Oberste MS, Maher K, Michele SM, Belliot G, Uddin M, Pallansch MA (2005) Enteroviruses 76, 89, 90 and 91 represent a novel group within the species Human enterovirus A. J Gen Virol 86:445–451
23. Norder H, Bjerregaard L, Magnius L, Lina B, Aymard M, Chomel JJ (2003) Sequencing of "untypable" enteroviruses reveals two new types, EV-77 and EV-78, within human enterovirus type B and substitutions in the BC loop of the VP1 protein for known types. J Gen Virol 84:827–836