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First epidemic of aseptic meningitis due to echovirus type 13 among Spanish children

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SUMMARY

Echoviruses are the commonest cause of aseptic meningitis (AM). Echovirus type 13 (EV-13) was the second enterovirus serotype associated with different local outbreaks of AM in Spain between February and October 2000. It was the first time that an epidemic AM caused by this virus was recognized in Spain. The index case appeared in the Canary Islands (Canarias). The EV-13 virus was isolated from 135 patients, predominantly from cerebrospinal fluid (CSF). All isolates were from children under 13 years. The age specific peak incidence was in infants under 1 year. Most patients had fever, headache and other meningeal signs. This enterovirus serotype, not previously detected in Spain, caused severe illness with a high attack rate.

INTRODUCTION

The enteroviruses (EV) comprise a large genus, established in 1957, within the Picornaviridae family. Human EV were subclassified into poliovirus (PV, serotypes 1–3), coxsackievirus groups A (CAV, serotypes 1–22 and 24) and B (CBV, serotypes 1–6) and echoviruses (EV, serotypes 1–7, 9, 11–27 and 29–33) and the newer enterovirus serotypes 68–71 [1–3].

At present, only 64 immunologically distinct serotypes of all EV are known to cause infections in humans, because echoviruses 22 and 23 are not considered to be EV [3].

Examples of the acute diseases in which the aetiological role of EV is well documented are poliomyelitis, Bornholm disease, acute aseptic meningitis, encephalitis, acute myocarditis, diabetes mellitus type 1, orchitis and upper respiratory tract infections [4, 5]. Some patients die. However, most infections are mild or asymptomatic, especially in children. Severity depends on age and host factors as well as virulence of the circulating virus [6].

Aseptic meningitis (AM) is by far the most common of the clinically important infections in which EV are involved. Since the introduction of mumps vaccine, mumps meningitis has become far less common in those countries in which it is widely used. Accordingly, at least 85% of the cases of AM for which an aetiology can be determined, particularly children and infants, are now due to EV [7–9]. EV have a world-wide distribution but within a given geographical locality some serotypes may be endemic with little or only gradual change from year to year. In contrast, other serotypes may be introduced periodically, causing epidemics, with few isolations reported in intervening years [10, 11].

Epidemiological surveillance plays a crucial role in understanding the different patterns of EV infection and disease association. Such knowledge may finally help in the control of infectious diseases [12–15]. Although complete identification of EV does not contribute significantly to patient management, it is

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essential for epidemiological purposes, establishing the dominant virus each year or in each outbreak [13, 16].

In Spain, in recent years outbreaks of AM associated with non-polio EV have increased the size of the increase varies each year depending on the predominating serotype [11, 17, 18]. A higher than usual number of AM cases were notified in the year 2000 [19], the first time in 15 years in which EV-13 was detected and aetiologically associated with AM. Local outbreaks of AM occurred in different geographical Autonomous Communities of Spain and the Canary Islands.

In this paper, we present the virological and epidemiological results obtained after studying the AM outbreaks caused by EV-13.

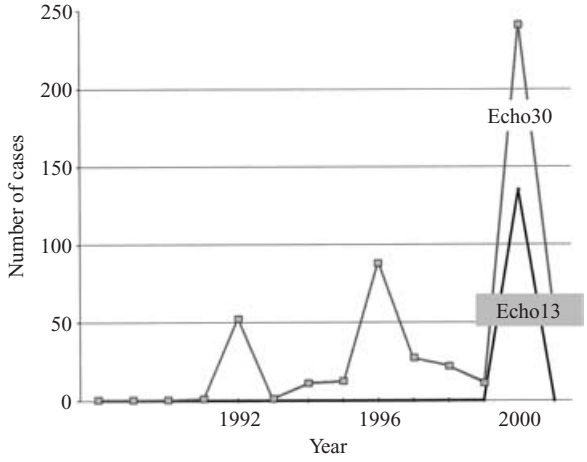

Fig. 1. Number of echovirus 13 and echovirus 30 isolates during the period 1988–2000 in Spain.

MATERIALS AND METHODS

During 2000, a total of 538 EV isolates from patients with AM (88%), fever (3·5%) or other diagnoses (8·5%) were received in the National Reference Laboratory for Poliomyelitis and Enteroviruses (CNM, ISCIII) from 15 different Spanish primary virological laboratories within 11 Autonomous Communities (AACCs). Viral isolation was performed on human rhabdomyosarcoma cells (RD), human embryonic fibroblast cells (HEF) and human lung carcinoma cells (A-549), selected for their support of EV replication [20, 21]. All isolates were studied by immunoflorescence using monoclonal mouse anti-enterovirus antibodies (clone 5-D8/1, Dako, Glostrup, Denmark).

The 538 EV isolates were serotyped by the standard method of micro-neutralization using both the Lim Beenyeesh–Melnick equine antiserum pools, supplied by the WHO Collaborating Centre for Virus Reference and Research pools [22], and the RIVM pools (National Institute of Public Health and the Environment, the Netherlands).

RESULTS

EV-30 was the predominant serotype accounting for 241 (45%) of 538 EV isolates in 2000. It is one of the commonest echovirus types isolated by laboratories and produces outbreaks every few years. Outbreaks of AM associated with EV-30 in Spain occurred in 1992, 1996 and have been reported by Trallero et al. [11] (Fig. 1).

EV-13 was the second most prevalent serotype, with 25% of the cases (135/538) during 2000. The 135 EV-13 isolates were recovered from RD cells (76%), from HEF cells (22%) and from other cells (2%). This serotype was found in several geographical AACCs of Spain (Fig. 2). The temporal presentation and geographical distribution of cases suggests the following pattern of spread. The index case of AM due to EV-13 appeared in February 2000. Subsequently, EV-13 started to circulate in the Canary Islands, where the highest number of cases occurred, 46% of patients (62/135). The virus persisted in this area until the end of the outbreak (October), with the highest incidence in April and May when 20 EV-13 isolates were reported each month. The virus was found later in the rest of AACCs of Spain. The second and third AACC affected were País Vasco and Madrid, with 17 and 12·5% of the total cases, respectively. The last cases were detected in October in Andalucía and Canary Islands. Cases were detected from February to October but most of them were diagnosed in June (34%), May (28%) and April (21%) (Table 1).

The 135 EV-13 isolates were from CSF (73%), stools (17%) and throat swabs (10%). Patients were affected with AM in 87% of cases in which EV-13 was identified, and the remaining 13% with a variety of clinical features (mainly fever, 5%). Of the affected individuals, 64% were male and 36% female. The patients were hospitalized in Canary Islands for a short period of time, ranging from 1 to 11 days, mean 4 days. The epidemic in this AACC generated 102 days of hospitalization and the outcome was good for all patients.

The age distribution of the EV-13 infections ranged from newborn to 13 years, with the age specific peak incidence in infants under 1 year of age. The mean age was 5 years. The range of ages of the EV-30 infections

Table 1. *Temporal distribution of echovirus 13 in the Autonomous Communities of Spain during the year 2000*

	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Total
Murcia	0	0	0	0	0	1	0	0	0	1
Aragón	0	0	0	0	2	0	0	0	0	2
Andalucía	0	0	0	0	1	1	0	0	1	3
Cantabria	0	0	1	2	0	0	0	0	0	3
Castilla León	0	0	0	1	2	0	0	0	0	3
Cataluña	0	0	2	1	2	0	0	0	0	5
C. Valenciana	0	0	2	5	0	0	0	0	0	7
Galicia	0	0	0	3	4	1	1	0	0	9
Madrid	0	4	1	4	8	0	0	0	0	17
Pais Vasco	0	0	2	2	14	5	0	0	0	23
Canarias	2	1	20	20	13	4	1	0	1	62
Total	2	5	28	38	46	12	2	0	2	135

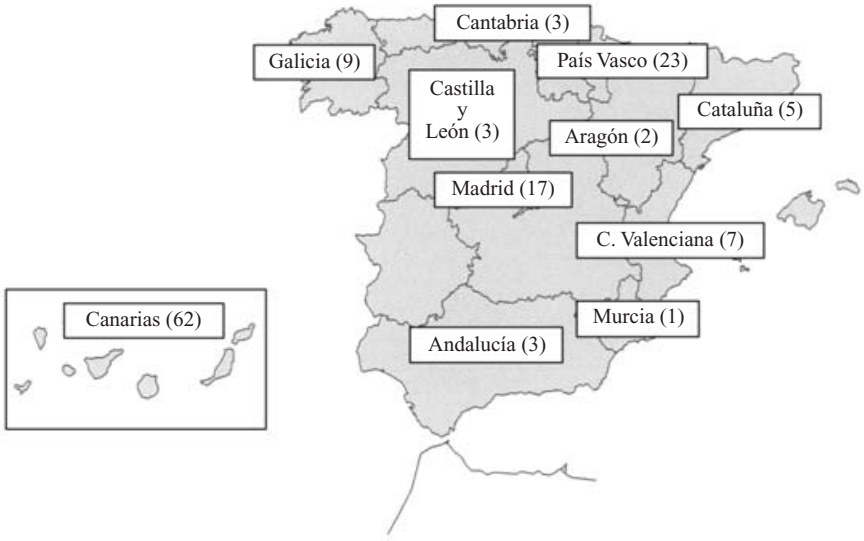

Fig. 2. Number of echovirus 13 isolates (135) in different Autonomous Communities of Spain in the year 2000.

was higher, from 1 to 80 years and this virus infected every group of age in contrast to EV-13 (Fig. 3). A high rate of AM was produced by both EV-30 and EV-13 viruses. In the EV-30 infections meningitis was diagnosed in the 97% of patients, affecting in similar proportion both males (49%) and females (51%). EV-13 affected males in a higher percentage (64%).

EV-30 and EV-13 infections were widely distributed in Spain and occurred in the same AACC. However, the number of affected people was very different in each AACC. Whereas EV-13 was responsible for 46% (62/135) of the cases in the Canary Islands, EV-30 was predominant in País Vasco, 45% (109/241), Cantabria 19%, Madrid 13%, and only 2% (5/241) in the Canary Islands.

At the same time as the Spanish outbreak, EV-13 caused other outbreaks in England and Wales, Scotland, Ireland, Germany, France and The Netherlands in 2000 (Table 2) [23].

DISCUSSION

Identification of EV-13 was performed by both immunoflorescence and standard methods of microneutralization. Since both methods have been used in our laboratory for over 15 years, we conclude that 2000 was the first year with evidence that EV-13 circulated in Spain. Although 135 isolates of EV-13 were typed, the number may be much higher. For example, in Canary Islands there were about 90 further EV isolates from patients with AM which were not typed but were linked to other patients with AM caused by EV-13. Of the 135 EV-13 typed, 87% were from the paediatric population.

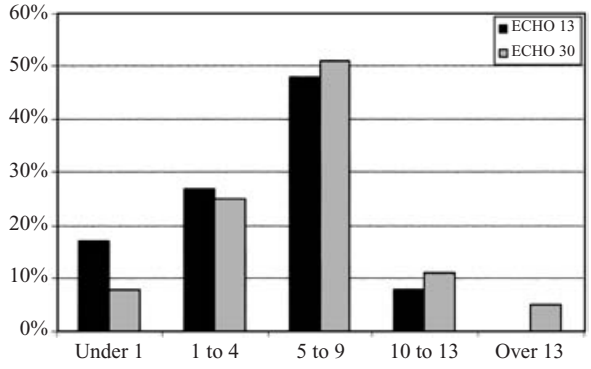

Fig. 3. Age distribution of echovirus 13 and echovirus 30 cases, 2000.

The age of patients associated with an EV-30 infection during 2000 on the other hand, ranged from 1 month to 80 years old with a mean of 7 years (Fig. 3). The age distribution of patients with EV-13 indicates that young children were at highest risk for infection. This could be because EV-13 did not circulate in Spain for at least 15 years and then was capable of causing widespread infections in young people because they had not been in contact with it before. This supports the view that EV, which were not epidemic during a 20 year period, still have the potential to cause a large epidemic [24, 25].

The use of a positive CSF specimen as an indicator of meningitis has the advantage of providing a consistent case definition [25]. We obtained viral cultures from CSF in most patients compared to others who reported a higher rate of positives from stools than CSF [26]. This may be because we collect CSF in the hospital 24–48 h after diagnosis of meningitis. Kajiwara [27] reported that viruses can be isolated from CSF obtained within 3 days of onset of the acute illness. The highest incidence of EV-13 occurred in June and May, a period of traditionally high incidence of EV infection in Spain, but the reported outbreaks of AM associated with this serotype started early in the EV season (March, April).

Our data included EV isolates from 15 different primary laboratories, 9 of them belonging to the laboratory network for AFP Surveillance, distributed across Spain. For this reason, we think that EV were evenly distributed throughout the country. Nevertheless, the number of EV isolates corresponding to each laboratory depended on the capacity and the interest of the laboratory.

EV-30 is known to be out of the most frequent enteroviruses causing outbreaks of sporadic cases of AM worldwide [25, 28]. Compared to previous years, there was an increase of AM in Spain in year 2000 [11, 19] and EV-30 was again the most prevalent serotype identified (45%, 241/538). Only a few serotypes, EV-30, Ev-9 and EV-6, appear to circulate endemically and others circulate in a cyclical fashion with epidemic years followed by years with decreased activity [7, 24, 25, 29, 30]. An example of this situation is EV-4 which was associated with all AM outbreaks that occurred in Spain during 1991 [11]. A similar epidemic behaviour seems to have been displayed by EV-13 during the year 2000 because it was detected in other European countries and outbreaks also occurred in 2001. For instance, in England and Wales a total of 4405 echoviruses were isolated and typed during 1990–9 and only 25 (0·02%) were found to be type 13. However in the year 2000, 238 EV-13 were typed [23, 31]. In France, the EV-13 outbreak accounted for 32·1% of the 1262 EV identified in 2000. In the Netherlands, the number of isolations of EV-13 peaked in October 2000 and also in September and October 2001, where this virus accounted for 7% (31/734) of EV isolated in 2001 [23]. In Germany the first local outbreak of AM was notified in May 2000, but the number of identifications in the year 2001 was twice that in 2000, with a peak of enterovirus activity occurring 3 months later [23, 32]. Outside Europe, there also were reports of EV-13 in Australia and in USA. In USA, 76 cases of EV-13 infection were reported between January and August 2001, when this EV had rarely been detected before [23, 33].

These data suggest that outbreaks of some EV types were more widespread than previously realized. Outbreaks may occur not only in several countries within one continent but also in different continents. Thus,

Table 2. *Incidence of echovirus 13 during the years 2000 and 2001 in different countries*

	2001	2000
Belgium	2	—
Denmark	4	4
England and Wales	15	238
France	6	405
Germany	145	55
Iceland	0	11
Ireland	0	44
Netherlands	44	47
Scotland	0	54
Spain	0	135
Australia	12	—
USA	76	—

—, No data.

EV-13 cases were reported in February 2000 in England and Wales but they began to rise only in June reaching a plateau during most of July to October [34]. Eight EV-13 had been reported in 1999 in Ireland, whereas 44 were in 2000 [35] and other undiagnosed and unreported cases may have occurred elsewhere. Since the first documented case of EV-13 was detected in February in the Canary Islands, and this AACC is a tourist destination for many people, especially in winter, it is feasible to think that this strain was closely related to the Ireland strain, and may have been responsible for some of the outbreaks in Europe during 2000. Molecular studies to elucidate the spread of EV-13 are in progress.

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