Epidemiol. Infect. (1997), **119**, 71–77. Printed in the United Kingdom

Epidemiological features of entero non-poliovirus isolations in Belgium 1980–94

E. DRUYTS-VOETS*

Institute of Hygiene and Epidemiology, Juliette Wytsmanstraat 14, B1050 Brussels, Belgium

(*Accepted 13 February 1997*)

SUMMARY

Between 1980 and 1994 the virological laboratory of the Institute of Hygiene and Epidemiology was responsible for the typing of enteroviruses, isolated by other, mainly university, hospital laboratories. Altogether 3333 strains of entero non-polio (EnP) were typed. The number and the most common types of EnP virus isolations varied from year to year. Each year two to three predominant types determined the main character of the EnP associated illness seen.

INTRODUCTION

Enteroviruses are a group of 65 different agents causing illnesses that range from mild, non-specific manifestations to syndromes as severe as aseptic meningitis and permanent paralysis. A large proportion of enterovirus infection is subclinical and does not reach the attention of the physician. A single enterovirus serotype may produce a variety of syndromes, and a particular syndrome may be produced by a number of different enteroviruses as well as by members of other virus groups. For these reasons, clinical symptoms are not a satisfactory basis for diagnosis [1], and isolation and typing of viruses are important as an aid in the diagnosis and therapy of these illnesses.

For 15 years the virological laboratory of the 'Instituut voor Hygiëne en Epidemiologie' of the Ministry of Public Health in Belgium was a reference centre for the typing of enteroviruses, isolated by other, mainly university, laboratories. This report presents the enterovirus-typing and clinical features.

MATERIALS AND METHODS

Viruses

Virus strains were isolated by several laboratories, receiving samples from all over the country. Most strains were isolated from faeces, but we also received strains isolated from nasopharyngeal swabs, cerebrospinal fluids (CSF) and several other body fluids.

The following four cell types were used for propagation of the virus strains: RD (human rhabdomyosarcoma), MRC-5 (human diploid lung), HEp-2 (human epidermoid larynx carcinoma) and Vero (African green monkey kidney). All cultures were examined daily. RD and HEp-2 cultures were kept for 7 days after inoculation and, if no cytopathic effect (CPE) occurred, they were freeze-thawed and passaged on fresh cells. MRC-5 and Vero cells were kept for 14 days after inoculation. When CPE was 90–100%, the infected cells were harvested and kept frozen (−20 °C) until typing.

Typing procedure

The isolates of enteroviruses were typed by semi-micro seroneutralization using intersecting antiserum pools. Two sets of these enterovirus typing antiserum pools were used: (*a*) the Lim Benyesh-Melnick (LBM) antiserum pools, supplied by the WHO Reference Laboratory in Copenhagen; (*b*) the 'Horse serum for typing of Enteroviruses' made by J. G. Kapsenberg at the RIVM (Bilthoven, The Netherlands).

With these two sets of antiserum pools 18 types of coxsackie A viruses [2], the 6 types of coxsackie B

* Author for correspondence.

Table 1. *Patients with entero non-polio virus (EnP) isolation (n = 333) and meningitis patients (n = 790) by type of virus*

Virus type	EnP *n*	Meningitis *n*	Meningitis %	Virus type	EnP *n*	Meningitis *n*	Meningitis %
Coxsackie A2	12	1		Echo 1	24	1	
Coxsackie A3	2			Echo 2	4	2	
Coxsackie A4	23	1		Echo 3	108	5	4·6
Coxsackie A5	6			Echo 4	54	34	63·0
Coxsackie A6	9			Echo 5	72	21	29·2
Coxsackie A7	1			Echo 6	225	33	14·7
Coxsackie A8	6			Echo 7	175	32	18·3
Coxsackie A9	93	24	25·8	Echo 9	73	41	56·2
Coxsackie A10	19			Echo 11	282	56	19·9
Coxsackie A11	6			Echo 12	7	1	
Coxsackie A13	8	3		Echo 13	35	4	
Coxsackie A14	5			Echo 14	64	4	6·3
Coxsackie A15	14	1		Echo 15	22	3	
Coxsackie A16	43	1	2·3	Echo 16	15	3	
Coxsackie A17	3			Echo 17	30	6	
Coxsackie A18	3			Echo 18	51	8	15·7
Coxsackie A20	6			Echo 19	16	1	
Coxsackie A21	9	1		Echo 20	103	28	27·2
				Echo 21	71	13	18·3
Total	268	32	11·9	Echo 22	169	7	4·1
				Echo 23	5		
				Echo 24	30	10	
Coxsackie B 1	127	15	11·8	Echo 25	122	12	9·8
Coxsackie B 2	133	16	12·0	Echo 27	4	2	
Coxsackie B 3	165	10	6·1	Echo 29	8		
Coxsackie B 4	120	16	13·3	Echo 30	389	220	56·6
Coxsackie B 5	199	36	18·1	Echo 31	7	3	
Coxsackie B 6	1			Echo 32	1		
				Echo 33	154	115	74·7
Total	745	93	12·5				
				Total	2320	665	28·7

viruses and 29 types of echovirus can be differentiated [3], all of them growing in the cell cultures mentioned above. Some typings were verified by neutralization with monospecific antisera from the American Type Culture Collection.

Patients with stated symptoms of meningitis and the patients with EnP virus isolation from CSF were considered as meningitis patients. Infections occurring during the first 2 months of life were considered as early infant infections.

RESULTS

Echoviruses represented 69·6% of EnP virus isolations (Table 1), with, in decreasing order, echo 30, echo 11, echo 6, echo 7 and echo 22 as the most frequently isolated virus types. Coxsackie B viruses (mainly types 5 and 3) accounted for 22·4% of isolations. Eight per cent of EnP virus isolations belonged to the coxsackie A group (mainly types 9 and 16).

Fifteen of 53 EnP virus types identified using the antiserum pools represented 76·9% of all isolations (between 55·4 and 92·4% each year) (Table 2). Echovirus type 30 was the most frequently isolated EnP virus during these 15 years, with peaks in 1980, 1988 and 1992. Coxsackie B5 virus was isolated in more than 10% of the EnP isolations every 3 years. Echovirus type 22 had two peak years: 1990 and 1991. Most striking, however, was the echovirus type 33 epidemic of 1982: it represented 57·6% of the virus isolations during that year. Each year 18–38 different types of EnP virus were isolated (mean 29), with 2 or 3 predominant types determining the main character of the EnP associated illness seen.

https://doi.org/10.1017/S0950268897007656 Published online by Cambridge University Press

Table 2. *The 15 most common types of EnP viruses isolated in Belgium (1980–94). Percentage of total patients with EnP virus by year*

	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	Total
Echo 30	38·7	5·8	1·7	3·4	4·0	2·9	1·4	9·1	31·3	5·5	0·9	4·8	41·2	6·7	2·5	11·7
Echo 11	3·6	12·9	3·4	14·9	9·4	3·9	13·6	8·4	13·0	2·5	5·1	13·0	2·5	21·5	3·8	8·5
Echo 6	8·1	14·8	3·0	6·1	9·4	2·0	5·2	18·5	5·3	1·8	3·2	11·7	9·7			6·8
Coxsackie B5	11·7	9·0	3·0	10·1	1·3	7·8	13·6	5·6	2·5	10·1		0·9	14·1		1·3	6·0
Echo 7		5·5	1·3	2·7	10·3	17·1	0·9	1·7	2·8	8·9	16·7	1·3	2·5	4·7	1·3	5·3
Echo 22		1·3	0·8	2·7	0·9	5·9	7·0	3·5	2·8	4·6	12·5	18·3	6·5	5·4	0·6	5·1
Coxsackie B3	3·6	0·6		4·7	1·8	12·2	0·5	15·4	0·8		6·9	1·7	0·4	13·4	22·9	5·0
Echo 33		1·3	57·6	0·7			0·9	0·7	1·0			3·0				4·6
Coxsackie B2	5·4	1·9	1·3	0·7	9·8		0·9	0·7	8·0	1·2	6·0	7·0	5·4	8·7	0·6	4·0
Coxsackie B1		14·2	1·3	3·4	3·1		0·5	0·3		10·4	19·4		0·4	3·4	3·8	3·8
Echo 25	2·7		0·4	4·7	1·3	9·3	8·0	0·7	1·5	6·4	2·3	4·3	0·4	1·3	15·9	3·7
Coxsackie B4	4·5	3·2	3·0	1·4	0·9	3·9	4·7	2·8	2·3	7·4	1·4	7·4	1·8	8·1	1·9	3·6
Echo 3	0·9		3·0		0·9	6·8	6·1	1·4	3·8	10·7		0·4	0·4	10·1		3·2
Echo 20		1·9			0·4			1·0	7·3	3·4	0·9	2·6			30·6	3·1
Coxsackie A	2·7	2·6	1·7	4·7	1·8	1·5	4·7	0·7	2·5	2·5	7·4	0·9	3·2		7·0	2·8
Total	82·0	74·8	81·4	60·1	55·4	73·2	68·1	70·6	84·5	75·5	82·9	77·4	88·4	83·2	92·4	76·9
Total no. of EnP patients *n*	111	155	236	148	224	205	213	286	400	326	216	230	277	149	157	3333
No. of meningitis patients	27	27	134	43	41	32	47	39	111	51	23	39	118	32	26	790

More than 20% of patients infected with this virus type.
Between 10 and 20% of patients infected with this virus type.
Year of meningitis epidemic.

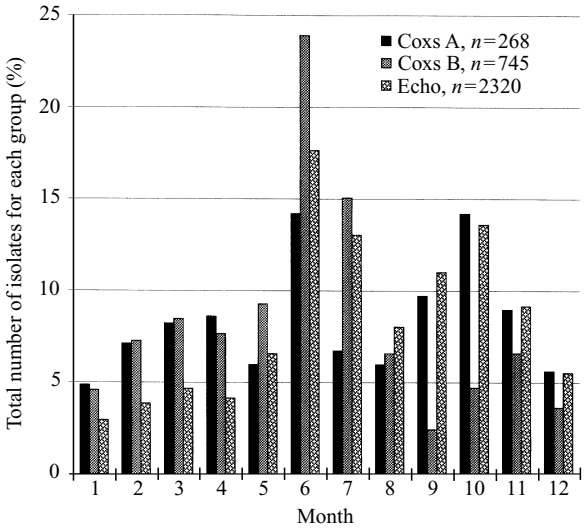

Fig. 1. Monthly distribution of entero non-polio virus (EnP) isolations (%).

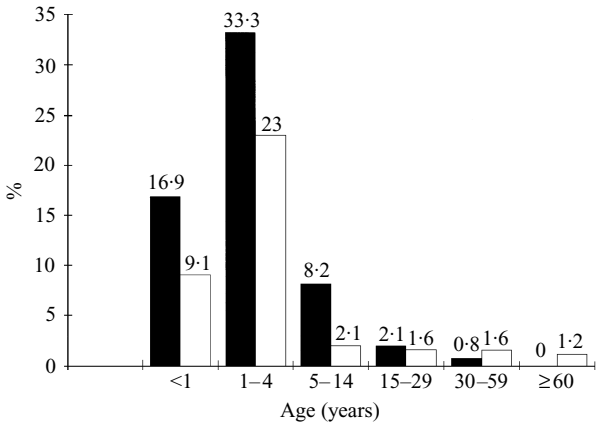

Fig. 2. Distribution of patients with Coxsackie A virus isolation according to age and sex. $n = 243$. ■, Male (61·3%); □, female (38·7%).

Coxsackie A viruses were present throughout the year, showing small peaks in June and October (Fig. 1). Coxsackie B viruses peaked in June. Echoviruses showed two peak periods: June–July and September–October.

Coxsackie A and B viruses were isolated mainly from children aged 1–4 years (A: 56·3% and B: 52·1%) with a male:female ratio of about 2:1 (Figs. 2, 3). Echovirus were isolated mainly from children under 1 year (Fig. 4).

Meningitis

Three meningitis epidemics were observed during these 15 years of EnP typing: in 1982, 1988 and 1992 with 134, 111 and 118 cases (Table 2, Fig. 5). They all

https://doi.org/10.1017/S0950268897007656 Published online by Cambridge University Press

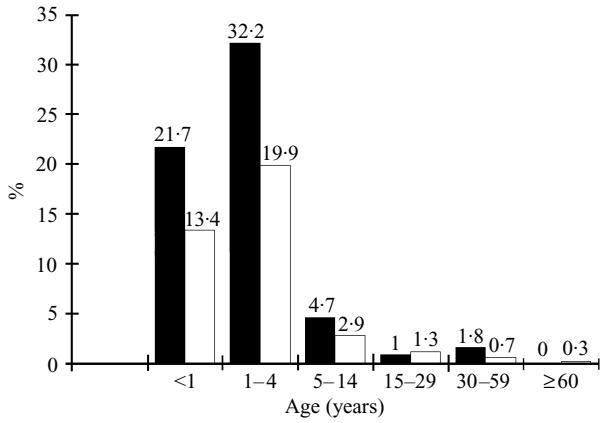

Fig. 3. Distribution of patients with Coxsackie B virus isolation according to age and sex. $n = 678$. ■, Male (61·4%); □, female (38·6%).

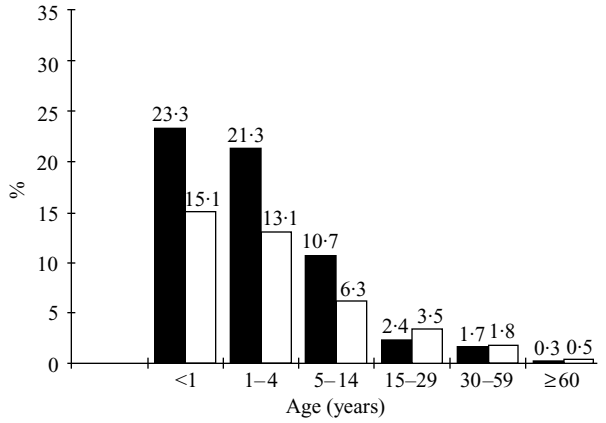

Fig. 4. Distribution of patients with echovirus isolation according to age and sex. $n = 2116$. ■, Male (59·7%); □, female (40·3%).

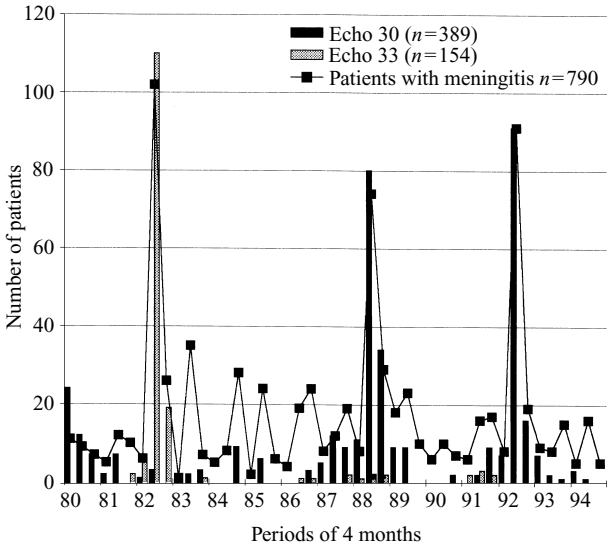

Fig. 5. Distribution of patients with meningitis and patients with echovirus types 30 and 33 isolation per period of 4 months (1980–94).

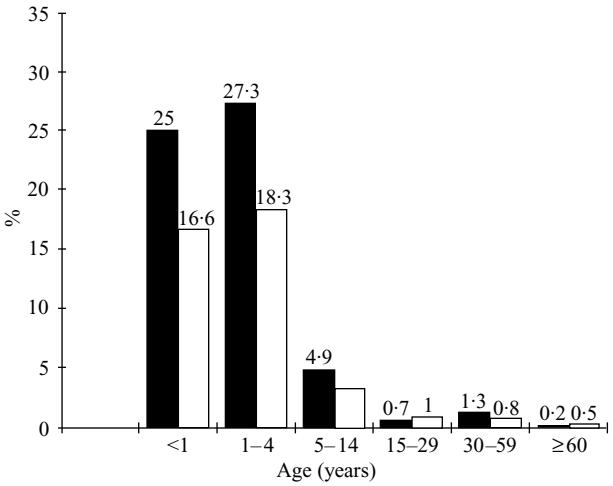

Fig. 6. Distribution of patients with EnP virus isolation, without meningitis patients, according to age and sex. $n = 2320$. ■, Male (59·4%); □, female (40·6%).

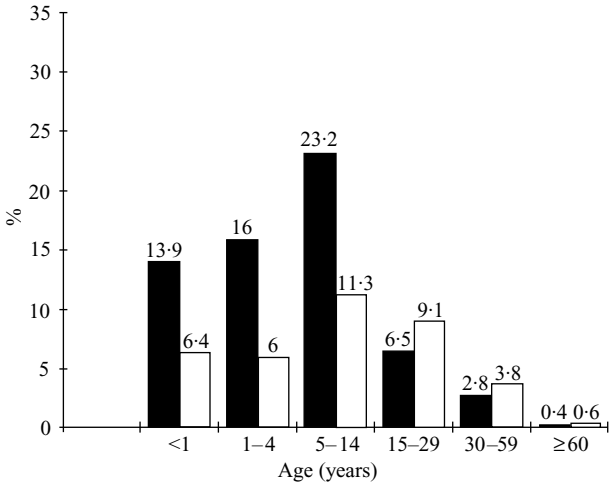

Fig. 7. Distribution of meningitis patients according to age and sex. $n = 717$. ■, Male (62·9%); □, (37·1%).

occurred during the sumer months (June and July). The peak in 1982 was due to echovirus type 33, and the peaks of 1988 and 1992 to echo 30 virus.

Certain echovirus types appear to be associated more often with symptoms of meningitis (Table 1): echo 9 (56·2%), echo 30 (56·6%), echo 4 (63·0%) and echo 33 virus (74·7%).

The age and sex of patients with EnP virus isolation, who did not have meningitis ($n = 2543$, 2320 with data about age and sex), are shown in Figure 6. Most (87·3%) of the isolations occurred in children under the age of 5 years. By contrast, in the group with meningitis, there was a shift to the higher age groups (Fig. 7). Two-thirds of all meningitis patients were boys.

https://doi.org/10.1017/S0950268897007656 Published online by Cambridge University Press

Early infant infections

On average, 10·2% (between 6·8 and 12·9%) of the EnP virus isolations were from this group. Echo 22, coxsackie B1 and echo 25 viruses were the virus types most frequently found.

DISCUSSION

Enteroviruses and adenoviruses are often present in faeces of normal children under 5 years of age [4], so that the results of routine laboratory investigations have to be interpreted cautiously [5].

In the Northern hemisphere, infections of all EnP virus types are least common during winter and spring months. Incidence increases rapidly in summer, although in the larger outbreaks reported cases continued to occur in winter [5–8]. Our survey confirms this pattern of incidence, but draws attention to the difference between coxsackie A viruses, with two peak months, June and October, coxsackie B viruses with only one peak month, June, and echoviruses, which were predominant from June until October. The prevalence of EnP virus in surveys conducted since 1967 (see Table 3) has shifted: coxsackieviruses (A + B) are less common and echoviruses more common. This can partly be explained by differences in the cell cultures used and the diminishing use of new-born mice for isolation. The introduction of RD cells however has increased the isolation rates of coxsackie A and echoviruses [8, 12, 13].

Studies in Glasgow [5] showed the cyclic recurrence of eight virus types over 20 years of observation. Although in any one year 25 or more different echovirus types were isolated in the Regional Virus Laboratory, some virus types showed a tendency to recurrence at intervals of 4 years (e.g. echovirus 9) or longer (echovirus 4, 30). In our survey different patterns in the presence of virus types became evident; echo 30, echo 11, echo 6 (until 1992), coxsackie B3, coxsackie B5 with outbreaks every 3 years; coxsackie B2, coxsackie B4, coxsackie A9, were endemic and did not cause outbreaks; echo 33, and echo 20 occurred only in outbreaks.

Moore [7] found that most reported cases of enteroviral disease in the US occurred in young children: 56% were under 10 years of age and 26% were under 1 year. Adults 20 years of age and older accounted for 16–20% of all the patients with non-polio enteroviral isolates. In our study children represented 91% of the total. Coxsackie A and B infections were most frequent in the 1–4 age group and echoviruses in the < 1 year age group.

Reported isolations of enteroviruses were consistently more common from males than from females. The reason remains unclear. We can confirm the statement of Moore [7] that the predominance of male patients is seen even among children under 1 year of age, when social behaviour and handling by parents are likely to be similar for males and females. There were large differences in the age distribution of different virus types.

During the 15 years of observation, comparable epidemics of viral meningitis occurred in the surrounding countries: in France in 1982 with echo 33 virus, which was demonstrated by virus isolations and detection of specific ELISA IgM [14, 15], and in The Netherlands in 1982 (with echovirus type 6 and 33) and in 1988 with many proven echovirus type 30 infections [16].

Serious neonatal infection is associated with specific echovirus serotypes (especially echovirus 11), male sex, prematurity, and prepartum maternal illness. Our study confirms the importance of echovirus in early infancy [17]; 73·6% of neonatal infections were related to echoviruses and 22·6% to coxsackie B viruses. Several types of echovirus are involved: type 4 in febrile illnesses, type 9 in fatal or mild infections, type 11 in outbreaks of fever, meningitis, respiratory or gastrointestinal illnesses [1, 5]. Neonatal fatalities caused by echoviruses have been reported, mainly involving echovirus 11, but also the serotypes 6, 7, 12, 19, 21 and 31 [18]. Our results support the importance of echo 11 virus, but also echo 22 virus and coxsackie B1, in neonatal infections, followed by echo 6, echo 25 and coxsackie B5.

During 15 years we have noted two outbreaks of coxsackie B virus infection in a maternity unit [19], in 1988 (coxsackie B2 virus), and 1989 (coxsackie B1 virus) and one outbreak of echovirus type 20 in 1994 (unpublished results). In each of the three outbreaks the isolated virus types were the most prevalent serotypes in the general population. During the outbreak of 1994 echovirus type 20 was isolated from different samples of six neonates, all of them males, born in different towns (Gent (1), Brussels (1), Antwerpen (4)). Two of them died. Our conclusion is that when outbreaks of EnP viruses occur in the general population, neonates are specially at risk. Epidemiological data can therefore become important in attempting to minimize the risk of neonatal

Table 3. *Distribution of the EnP virus isolations over the three groups* (%) *and enumeration of the most frequently isolated types: comparison of data from different authors*

				Coxsackie A		Coxsackie B		Echo	
Author	Population	Period	*n*	%	Types	%	Types	(%)	Types
Assaad and Cockburn (6)	Worldwide WHO data	1967–70	16,465	13·6	9, 16	34·1	3, 2	52·3	9, 6, 30
Grist *et al.* (5)	Worldwide WHO data	1967–74	41,540	12·7	9, 16	31·9	5, 3	55·4	9, 6, 11, 30
Moore (7)	USA	1970–79	18,152	8·9	9	26·7	5, 2, 4, 3	64·4	9, 11, 4, 6, 7, 3
Strikas (9)	USA	1970–83	23,481	—	9	—	5, 2, 4, 3	—	11, 9, 30, 4, 6, 3
Grist and Reid (10)	Worldwide WHO data	1975–83	58,912	9·8	9	25·4	4, 2, 3	64·8	—
Weber (11)	Canada	1980–88	—	—	9	—	5, 4	—	7, 9, 11, 22
Present data	Belgium	1980–94	3,333	8·0	9, 16	22·4	5, 3	69·6	30, 11, 6, 7, 22

infections, by following the guidelines for preventive measures of enteroviral infections at delivery, as described by Magnus [18].

The epidemiological features of type 22 echovirus infections differed from most enterovirus infections with regard to age and seasonal distribution, contagiousness and relative lack of protection by neutralizing antibodies. Most virus isolations were made from children under 1 year of age (88·3%), associated in neonates with respiratory (14·6%) and diarrhoeal (56·2%) illness. The seasonal distribution also showed a difference from the other EnP viruses, with the presence of the echo 22 virus during the winter period (from August until March) and a decline in the month of June. Our data confirm the data of Ehrnst & Eriksson [20], which lend support to the conclusion that echovirus 22 belongs to a separate subgroup of picornaviruses.

ACKNOWLEDGEMENTS

We appreciate the comments of Roland Dobbelaer and Geneviève Ducoffre on the manuscript; and we acknowledge with thanks the different virus laboratories who collected the enterovirus strains and sent them to our laboratory for typing (Universitaire kliniek St Rafaël, Leuven; Universitair Ziekenhuis Gent; Universitair Ziekenhuis Antwerpen; Dokter Willems Instituut, Diepenbeek; Universitaire Ziekenhuizen Brugmann, St Pieter and Jette, Brussel; Ecole de Santé Publique, Woluwe; Akademisch Ziekenhuis Middelheim, Antwerpen; Laboratorium Medische Analyse, Zwijnaarde).

REFERENCES

1. Melnick JL. Enteroviruses. In: Evans AS, ed. Viral infections of humans. Epidemiology and control, 2nd ed. New York: Plenum Press, 1982: 187–251.
2. Melnick JL, Schmidt NJ, Hampil B, Ho HH. Lyophilized combination of pools of enterovirus equine antisera: preparation and test procedures for the identification of field strains of 19 group A coxsackievirus serotypes. Intervirol 1977; **8**: 172–81.
3. Lim KA, Benyesh-Melnick M. Typing of viruses by combinations of antiserum pools. Application to typing of enteroviruses (coxsackie and ECHO). J Immunol 1960; **84**: 309–17.
4. Galbraith NS. A survey of enteroviruses and adenoviruses in the faeces of normal children aged 0–4 years. A report of the Public Health Laboratory Service and the Society of Medical Officers of Health. J Hyg 1965; **63**: 441–55.
5. Grist NR, Bell EJ, Assaad F. Enteroviruses in human disease. Prog Med Virol 1978; **24**: 114–57.
6. Assaad F, Cockburn WC. Four-year study of WHO virus reports on enteroviruses other than poliovirus. Bull WHO 1972; **46**: 329–36.
7. Moore M. Enteroviral disease in the United States, 1970–1979. J Infect Dis 1982; **146**: 103–8.
8. Glimåker M. Enteroviral meningitis. Scand J Infect Dis 1992; suppl. **85**: 1–60.
9. Strikas RA, Anderson LJ, Parker RA. Temporal and geographic patterns of isolates of nonpolio enterovirus in the United States, 1970–1983. J Infect Dis 1986; **153**: 346–51.
10. Grist NR, Reid D. General pathogenicity and epidemiology. In: Bendinelli M, Friedman H, eds. Coxsackieviruses. New York: Plenum Press, 1988; 221–39.
11. Weber JM, Parker CA. Laboratory diagnosed human viral infections in Canada, 1980–1988: trends and clinico-epidemiological characteristics. Diag Microbiol Infect Dis 1991; **14**: 225–32.

12. Schmidt NJ, Ho HH, Lennette EH. Propagation and isolation of group A coxsackie viruses in RD cells. J Clin Microbiol 1975; **2**: 183–5.
13. Druyts-Voets E, Yane F, Bosmans E, Colaert J, Desmyter J. Method for selecting optimal cells for enterovirus isolation as determined in an outbreak of echovirus type 33 meningitis. Europ J Clin Microbiol 1985; **4**: 331–4.
14. Celers J, Netter R. Méningites lymphocytaires virales en France de 1978 à 1983. Analyse de résultats d'isolements de virus. Méd Malad Infect 1985; **10** bis: 612–5.
15. Chomel J, Thouvenot D, Fayol V, Aymard M. Rapid diagnosis of echovirus type 33 meningitis by specific IgM detection using an enzyme linked immunosorbent assay (ELISA). J Virol Meth 1985; **10**: 11–9.
16. van Loon AM, Ossewaarde JM, Netherlands Working Group on Clinical Virology. Laboratory diagnosis of viral meningitis and encephalitis in the Netherlands, 1981–1991. Analysis made for the 'Concerted action on viral meningitis and encephalitis'. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands, 1994; 1–12.
17. Modlin JF. Perinatal echovirus infection: insights from a literature review of 61 cases of serious infection and 16 outbreaks in nurseries. Rev Infect Dis 1986; **8**: 918–26.
18. Magnius L, Sterner G, Enocksson E. Infections with echoviruses and coxsackieviruses in late pregnancy. Scand J Infect Dis 1990; Suppl. **71**: 53–7.
19. Druyts-Voets E, Van Renterghem L, Gerniers S. Coxsackie B virus epidemiology and neonatal infection in Belgium. J Infect 1993; **27**: 311–6.
20. Ehrnst A, Eriksson M. Epidemiological features of type 22 echovirus infection. Scand J Infect Dis 1993; **25**: 275.