

An Outbreak of Aseptic Meningitis due to ECHO 25 Virus

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ABSTRACT

In a widespread outbreak of aseptic meningitis during the summer and early fall of 1961 in Ontario, ECHO type 25 virus was found to be the predominant virus type isolated from affected patients. Recovery of ECHO 25 virus in several cases from the CSF of patients strongly suggests an etiological relationship between this virus type and the illness. The causative role of the virus was confirmed by demonstration of significant immunological response in patients found to be infected with the virus. The presence of antibodies to ECHO 25 virus in about 70% of meningitis patients, from whom no virus was isolated, indicates that the infection in the population of the affected areas was much more prevalent than the number of successful virus isolations would suggest. It is recommended that ECHO 25 virus should be added to the series of ECHO virus types capable of causing outbreaks of aseptic meningitis.

SOMMAIRE

Au cours d'une vaste épidémie de méningite aseptique, survenue en été et au début de l'automne 1961 en Ontario, on a constaté que le virus du type ECHO 25, isolé chez des malades, était le virus prédominant. Il existe une forte présomption qu'il y a une relation étiologique causale entre la maladie et le virus ECHO 25, ce dernier ayant pu être découvert dans le LCR de plusieurs malades. Le rôle étiopathologique de ce virus a été confirmé par la forte réaction immunologique que présentaient des malades qui étaient atteints de ce virus. Par ailleurs, la présence d'anticorps au virus ECHO 25 chez près de 70% des malades souffrant de méningite, où on n'avait pu isoler le dit virus, est une indication que l'épidémie avait fait plus de ravages dans la population de cette région que ne la laissait supposer le nombre de cas où il avait été possible d'isoler le virus. On conseille donc d'ajouter le virus ECHO 25 à la série de virus du type ECHO susceptibles de provoquer un épidémie de méningite aseptique.

VIRUSES most frequently isolated from cases of aseptic meningitis are poliovirus, Coxsackie and ECHO viruses. Of the 32 recognized types of the ECHO virus group, so far only a few have been proved to be responsible for definite outbreaks of aseptic meningitis. The most common of these offenders are types 4, 6 and 9;¹ those encountered with less frequency are types 7,^{2, 3} 11,^{4, 5} and 16.^{6, 7} Recently, type 30 (Frater-virus) was shown to be associated with distinct outbreaks of meningitis in Scotland,⁸ Canada⁹ and the United States.¹⁰⁻¹² Several other types,¹³ among them ECHO type 25 virus, were encountered in sporadic cases only. Ozere, Faulkner and van Rooyen¹⁴ reported isolation of ECHO 25 virus in 1958 on two occasions, from Newfoundland. In both cases the isolates originated from fecal specimens of meningitis patients.¹⁵ In California, Schmidt *et al.*¹⁶ recovered this virus type from the stool of one patient with non-specified "central nervous system disease" and demonstrated significant serological response to the virus in two-phase sera. Since its recognition, ECHO type 25 virus has been known in general to be associated with mild upper respiratory infections.¹⁷⁻¹⁹

The following communication deals with the etiological role of ECHO type 25 virus in a widespread outbreak of aseptic meningitis in Ontario.

MATERIALS AND METHODS

Specimens examined were stools, and swabs, throat washings, throat swabs, cerebrospinal fluid (CSF) and blood submitted to our laboratory for virus studies by practising physicians and hospitals from patients residing in various localities in the Province of Ontario. Methods of collection, shipment, storage and preparation of specimens were described in a previous communication.²⁰

For isolation and identification of viruses as well as antibody assays, monolayer cultures of trypsinized Rhesus monkey and human amnion (FL) cells were employed, using standard techniques. FL cell cultures were found to be more sensitive for both the isolation and typing of ECHO 25 virus isolates.

RESULTS

Virus Isolations

During the summer and early fall of 1961 there was a widespread outbreak of aseptic meningitis

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Fig. 1.—Geographical origin of ECHO 25 virus isolates.

involving 13 different localities scattered throughout the densely populated southern part of Ontario (Fig. 1). Specimens for virus isolation were received from 121 patients residing in these areas and for serological studies from an additional 57 cases. The results of virus isolations are listed in Table I. Successful isolations were made from

TABLE I.—VIRUS ISOLATIONS FROM CASES OF ASEPTIC MENINGITIS DURING JULY-OCTOBER, 1961, IN ECHO 25 VIRUS AFFECTED AREAS

	Type of virus isolates						Not yet identified	Total
	Coxsackie B			ECHO				
	2	4	5	9	14	25		
No. of cases....	2	1	7	10	1	29	6	56
Per cent.....			12.5	17.9		50.1		

specimens from 56 patients. ECHO 25 virus was recovered on 36 occasions from 29 (50.1%) of these patients. Coxsackie B5 and ECHO 9 infections accounted for another 30% of the cases. The remaining isolates were two Coxsackie B2, one each of Coxsackie B4 and ECHO 14, and six unidentified viruses.

Source of ECHO 25 Virus Isolations

The most common source of ECHO 25 virus isolations was stool (Table II). From one patient

TABLE II.—SOURCE OF ECHO 25 VIRUS ISOLATIONS

No. of cases	Isolations made from		
	Stool	CSF	Throat-washing
24	+		
2	+	+	
2		+	
1	+		+
Total: 29			

the virus was isolated also from the throat washings. From two patients the virus was recovered from both the stool and CSF, and from another two, from the CSF only.

In Table III the results of ECHO 25 virus isolations are tabulated according to the dates of

TABLE III.—ONSET OF ILLNESS AND ECHO 25 ISOLATIONS

Source of isolations	Days after onset of illness and number of isolations made				Total
	2-5	6-10	11-16	Not known	
Stool.....	11	7	5	8	31
CSF.....	1	—	1	2	4
Throat washing	1	—	—	—	1
Total.....	13	7	6	10	36

collection of specimens. From these data it is apparent that the stool specimens served as a good source of the virus, in some cases over two weeks after onset of symptoms. The majority of isolations, however, were made from stool specimens collected in the early stage of the illness (within the first five days). It is of interest to note that in one case the virus was isolated from CSF two weeks after the onset of illness.

In six households ECHO 25 virus was isolated from two or three members of the family, who were ill simultaneously or successively. The ages of the patients yielding the virus ranged from six weeks to 29 years, most of the patients being pre-school and school-age children. All ECHO 25 virus isolations were made from specimens submitted during a four-month period from July to October, the peak months being August and September.

The main symptoms and signs, in cases where ECHO 25 virus was isolated, were fever, malaise, headache, stiff neck, myalgic pains and nausea or vomiting, as well as pleocytosis in the CSF (110 white blood cells per ml. in the average) accompanied, in most cases, by elevated CSF protein levels (over 40 mg. %).

Serological Studies

Paired sera were available from nine patients yielding the virus and a single convalescent phase specimen from an additional patient. The results of antibody assay performed on these sera are presented in Table IV. Patients from whom two-phase sera were received revealed a significant rise in antibody to the isolate in their convalescent serum

TABLE IV.—SEROLOGICAL RESPONSE TO ECHO 25 VIRUS IN PATIENTS EXCRETING THE VIRUS

Sex	Age	Patient's Specimens	Titre of patient's serum	
			Acute	Con-valescent
Male.....	3	Stool	—	1 : 64
Female.....	6	"	—	1 : 128
Female.....	9	"	—	1 : 16
Male.....	12	"	—	1 : 128
Male.....	15	"	—	1 : 16
Male.....	19	"	—	1 : 32
Male.....	23	"	1 : 16	1 : 64
Male.....	26	"	—	1 : 32
Male.....	29	"	1 : 8	1 : 32
Female.....	29	CSF and stool	N.T.	1 : 64

Note: — = negative in dilution of 1 : 8.
N.T. = not tested.

samples. One patient from whom only a convalescent serum specimen was available, and whose CSF and stool specimens yielded the virus, had a titre of 1:64.

Further evidence of the prevalence of ECHO 25 virus infection in the population was obtained by testing paired sera of 101 patients with aseptic meningitis occurring during the same time period and in the same geographic areas. From about half of these patients no specimens were received for isolation studies and in the other half of these cases isolation attempts were unsuccessful. The results of this serological survey are summarized in Table V. A fourfold or greater increase in titre to ECHO 25 virus was found in paired sera of 29 patients. Sera of another 43 patients showed the presence of neutralizing antibodies to the virus at stationary levels. The remaining 29 patients proved to be free of antibodies to ECHO 25 virus.

TABLE V.—SEROLOGICAL RESPONSE TO ECHO 25 VIRUS IN PATIENTS NOT YIELDING THE VIRUS

No. of patients	Fourfold or greater increase in titre	Stationary titres in two-phase sera						Negative	Total
		1:8	1:16	1:32	1:64	1:128	1:256		
29	12	10	5	5	10	1	29	101	

Sporadic Occurrence of ECHO 25 Virus in Ontario

The first appearance of ECHO 25 virus in Ontario dates back to July of 1957, when this virus type was isolated in our laboratory from stool specimens of two children (sisters) living in Toronto. The virus was not encountered in Ontario during the years 1958, 1959 and 1960, and since the 1961 outbreak it was recovered only once, in September of 1962, from both the stool and CSF specimens of a patient with meningitis.

COMMENTS

During 1961, the most prevalent virus types associated with aseptic meningitis in the Province of Ontario as a whole²¹ were Coxsackie B5 and ECHO 9, with the exception of 13 localities where ECHO 25 accounted for 50% of the isolates.

The etiological significance of ECHO 25 virus in this outbreak of aseptic meningitis is strongly supported by the isolation of the virus from CSF and demonstration of immunological response in patients from whom the virus was isolated.

The results of the serological survey conducted on two-phase sera of meningitis patients from whom no virus was isolated suggest that the infection with ECHO 25 virus in the affected areas was much more prevalent than the number of successful isolations would indicate. Although there was a relatively large proportion of stationary titres found in paired sera, this could be attributed in many cases to the fact that the first serum samples were not collected early enough after onset of the illness and the

interval between the first and second specimens in other cases was too short.

Some antigenic differences were noted between the ECHO 25 isolates. These are being investigated and will be reported separately.

As far as the authors are aware, there are no reports in the literature associating ECHO type 25 virus with distinct outbreaks of aseptic meningitis. The data presented in this communication indicate that ECHO 25 virus, which in the past was implicated mainly in upper respiratory infections and on relatively rare occasions in sporadic cases of central nervous system infections, is capable of causing a widespread outbreak of aseptic meningitis.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

"CAUSES AND CURES OF CRIME"

This book expresses the opinions of one of the leading criminologists in America on crime and the criminal. These opinions are based on the premises that crime is, in most cases, the outcome of a diseased mind, that the prison should be reformed, and that the hospital should be more freely employed than the penitentiary. It is only fair to add that there is a contrary opinion that some men are criminals *in esse*, and that all men are criminal *in posse*. In the United States the cost of crime is given as one-third of the total cost of the government. It is seven times more prevalent in proportion to population than it was sixty years ago, and one in every thirty persons in the United States is defective or dependent. These facts are sufficient reason for this attempt to engage the public attention. The subject is large and extremely complex, and Mr. Mosby has brought to it his best thought. The author is a barrister, but he holds that the suppression of crime is not a legal question but rather a problem for physicians and economists. —Book Review, *Canad. Med. Ass. J.*, **4**: 228, 1914.