Coxsackie virus group B, type 5, widely distributed in the midwest during the summer of 1956, was highly prevalent in Iowa, with lower frequencies observed in Kansas, Missouri, and Nebraska. Concurrently in Iowa, the incidence of poliovirus infection was found to be extremely low.

Infections With Coxsackie Virus B5 in Six Midwestern States

TOM D. Y. CHIN, M.D., JOHN C. GREENE, D.M.D., and HERBERT A. WENNER, M.D.

THE RECOGNIZED viral agents responsible for the syndrome defined as benign aseptic meningitis include mumps, ECHO (enteric cytopathogenic human orphan), Coxsackie, poliomyelitis, and the arthropod-borne encephalitis viruses. Observations made during the summer and autumn months of 1956 indicated an unusually high incidence of cases of aseptic meningitis in certain midwestern communities. Fecal specimens submitted for poliomyelitis surveillance studies yielded a large number of isolates identified as Coxsackie virus, type B5.

The evidence that the B5 virus was etiologically related to many of the cases observed was reinforced by a singular study of a localized outbreak of aseptic meningitis caused by Coxsackie B5 virus in Cerro Gordo County, Iowa, during the same summer (*1*). The present report summarizes our experiences encountered with this virus with particular reference to differences in the distribution in the six midwestern States.

Materials and Methods

During the summer and autumn months of 1956, fecal specimens obtained from 694 individuals were studied in connection with the poliomyelitis surveillance program. Of this number, 365 were obtained from patients believed to have poliomyelitis; the remaining 329 specimens were collected from patients and family contacts during an investigation of an outbreak of aseptic meningitis. These specimens were from residents in the midwestern States of Arkansas, Iowa, Kansas, Missouri, Nebraska, and Oklahoma. In addition to the 694 fecal samples studied in the virus laboratory at the University of Kansas, the distribution study included 664 individual specimens also obtained from poliomyelitis patients, bringing the total of fecal samples to 1,358. The 664 specimens were studied in the State health department laboratories in Kansas and Missouri and in the virus laboratory of the University of Nebraska College of Medicine, results of isolations in these laboratories having been supplied by Dr. Charles A. Hunter, Irma C. Adams, and Helen W. Reihart.

Dr. Chin is assistant chief of the Kansas City Field Station, Communicable Disease Center, Public Health Service, where Dr. Greene serves as dental surgeon on temporary duty. Dr. Wenner is research professor of pediatrics at the University of Kansas Medical Center, Kansas City, Kans. Assisting in the study were Clifton R. Gravelle of the Kansas City Field Station and Vernon E. Scholes of the University of Kansas Medical Center.

Stool specimens were collected by practicing physicians or by the staff members in local hospitals. Usually single specimens were obtained. Fecal specimens were collected from household contacts only in connection with special investigation of localized outbreaks. The specimens were mailed unfrozen to the laboratory, where they were stored at −20° C. and held at this temperature until used.

The methods used for preparation of stools and for virus isolation and identification were those described by Wenner and Miller (*2*). Roller tube cultures of trypsin-dispersed monkey kidney cells prepared according to the methods described by Youngner (*3*) were routinely employed. Each specimen was tested in three culture tubes, using 0.1 ml. of stool extract per tube. The cultures were maintained for a 7-day period. Fluids of those cultures showing cytopathic changes were harvested for identification, using initially type-specific hyperimmune poliomyelitis antiserums (*4*). When the harvested fluid was not neutralized by each of the typing serums or by a combination of the serums, further identification was carried out with Coxsackle antiserums types 99, B1, B2, B3, B4, and B5, and with 14 types of ECHO antiserums. Neutralization was considered to have occurred if the culture tubes containing the virus-serum mixtures showed no cytopathic changes while cytolysis was present in the virus control tubes, and if this effect persisted for at least 2 additional days.

Virus Distribution in the Six States

Data on the frequency with which polioviruses and Coxsackie viruses were encountered in the specimens from 1,358 individuals submitted for study are summarized in table 1. The geographic distribution of individuals providing Coxsackie B5 viruses appears in figure 1.

The B5 virus was recovered from the stools of 270 individuals, and polioviruses, predominantly type 1, were recovered from the stools of 275. A stool from one individual yielded both poliovirus and Coxsackie B5 virus. Patients from Iowa provided a larger number of B5 viruses than those from the other five States studied. The recovery rates among 329 fecal

Table 1. Coxsackie virus B5 and poliovirus isolations from fecal specimens, by State, 1956

State	Number of persons	Coxsackie virus B5		Polioviruses	
		Number	Percent	Number	Percent
Arkansas	104	0	0.0	37	35.6
Iowa	329	173	52.6	8	2.4
Kansas	321	25	7.8	56	17.4
Missouri	424	51	12.0	141	33.2
Nebraska	165	20	12.1	29	17.6
Oklahoma	15	1	6.7	4	26.7
Total	1,358	270	19.9	275	20.2

samples from Iowa were about 53 percent for Coxsackie virus and 2.4 percent for polioviruses. In Kansas, Missouri, and Nebraska, the recovery rate for Coxsackie virus was approximately 11 percent and for polioviruses approximately 25 percent. In contrast with the experience in Iowa, Coxsackie B5 virus was not detected in fecal specimens from 104 individuals in Arkansas, although polioviruses were recovered from approximately one-third. The number of specimens originating in Oklahoma was too small for reliable comparison; nevertheless, one strain of Coxsackie B5 virus was recovered in the 15 fecal samples examined.

During the summer of 1956 aseptic menin-

Figure 1. Distribution of Coxsackie virus B5 in six midwestern States, 1956.

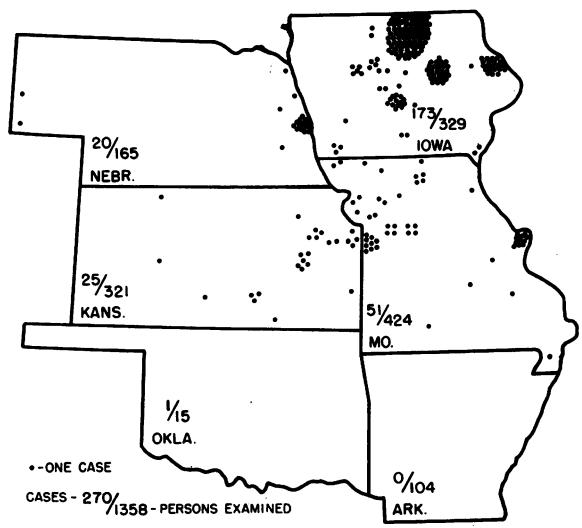

Table 2. Coxsackie virus B5 isolations from fecal specimens, by county, Iowa, 1956

County	Number of persons	Number positive	Percent positive
Cerro Gordo	135	82	60.7
Black Hawk	42	29	69.0
Dubuque	39	21	53.8
Polk	24	13	54.2
Webster	13	5	38.5
Calhoun	19	5	26.3
Others	57	18	31.6
Total	[1] 329	173	52.6

[1] Comprising 249 patients and 80 well persons.

gitis appeared in epidemic proportions in several localities in Iowa. The occurrence of this illness was first noted in Polk County during early July. In the middle of July similar illnesses were observed in Mason City, the county seat of Cerro Gordo County. Because the clinical features suggested that these illnesses were not caused by polioviruses, a detailed study was made in Cerro Gordo County in order to define the etiology and the clinical and epidemiological characteristics. Etiological studies indicated that these illnesses were caused primarily by Coxsackie virus, type B5.

The frequency with which Coxsackie B5 virus was encountered in different localities in Iowa appears in table 2. In Cerro Gordo County the virus was recovered from 82 of the 135 individuals examined. Of the 82 Coxsackie B5 strains recovered, 61 were isolated from 83 individuals with illness, while 21 were recovered from 52 well individuals, the majority being household contacts. A high frequency of virus recovery was obtained in the counties of Black Hawk, Polk, and Dubuque, whereas lower frequencies were observed in Webster and Calhoun Counties. Although extensive epidemiological investigation was conducted only in Cerro Gordo County, these data seem to indicate that a similar type of illness was also occurring in epidemic proportions in Black Hawk, Polk, and Dubuque counties.

The distribution of Coxsackie B5 viruses isolated from 144 Iowa patients by week of onset of illness appears in figure 2. The major number of isolations were made from patients who were ill during the months of August and September with the highest frequency occurring during the middle of August. A similar distribution was obtained for patients in Cerro Gordo County alone. In the State as a whole the seasonal distribution of Coxsackie B5 virus corresponded to that observed for poliomyelitis.

The distribution of Coxsackie virus B5 isolated from stool specimens of 329 Iowa residents analyzed according to age and health status is shown in table 3. Approximately 58 percent of the individuals who had an illness manifested as either aseptic meningitis or minor illness yielded the B5 virus, while about 36 percent of those with a history of no illness excreted B5 virus. In both ill and well groups the frequency of excretion was higher among those under 20 years than in those over 20 years of age.

Discussion

The first Coxsackie viruses were recovered by Dalldorf and Sickles in 1947 (*5*). During the past 10 years these viruses, now constituting a family of 24 members, have been encountered with an increasing frequency in association with a variety of illnesses. As a result of recent wide application of tissue culture techniques, Coxsackie viruses of types A9, B1, B2, B3, and B4 have been commonly recovered from patients with aseptic meningitis, pleurodynia, and myalgia, and occasionally

Figure 2. Coxsackie virus B5 isolations from feces of patients, by week of onset, 1956.

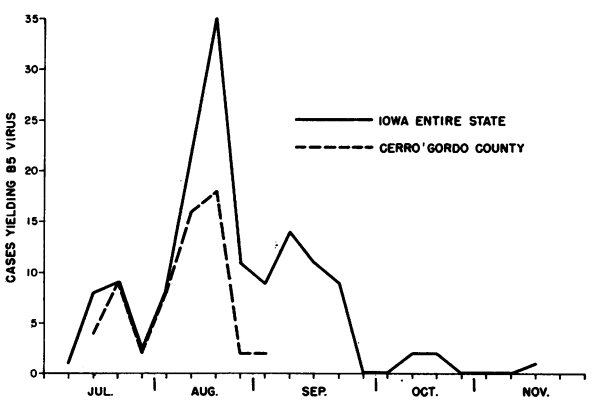

Table 3. Coxsackie virus B5 isolations from feces by age group and health status, Iowa, 1956

Age group (years)	Ill			Not ill [1]		
	Number of persons	Number positive	Percent positive	Number of persons	Number positive	Percent positive
0–4	51	35	68. 6	9	6	66. 7
5–9	68	40	58. 8	15	6	40. 0
10–14	42	27	64. 3	8	5	62. 5
15–19	17	10	58. 8	3	2	66. 7
20 and over	71	32	45. 1	45	10	22. 2
Total	249	144	57. 8	80	29	36. 2

[1] Comprising mostly household contacts.

from those with myocarditis. Until the summer of 1956, the type B5 Coxsackie virus, which was first isolated by Steigman in 1952 (*6*), had been found only occasionally. The data reported here and those recently reported by Syverton and associates (*7*) represent the first recognition of a widespread occurrence of this virus in association with human illness.

The variation in the geographic distribution of this virus in the six midwestern States is worthy of comment. Obviously, this virus appeared in epidemic proportions in Iowa. Of the 329 Iowans studied, about 53 percent yielded the B5 virus, while only about 2 percent yielded polioviruses. In contrast, none of the stools from 104 patients in Arkansas yielded Coxsackie viruses, while 36 percent were shown to contain polioviruses. In Kansas and Missouri, polioviruses were more commonly encountered than the B5 virus, while both viruses appeared in about equal frequency in Nebraska. The number of specimens obtained from Oklahoma was too small for reliable comparison.

Suggested as a factor producing variations in geographic distribution of Coxsackie B5 virus is the apparent incompatibility of Coxsackie B and poliovirus infections. In the laboratory Coxsackie B infections appear to exert an interfering effect on poliovirus infections in mice (*8*, *9*) as well as in tissue cultures (*10*). Field observations also suggest that such interference may occur in man, and the evidence in favor of this hypothesis has recently been reviewed by Dalldorf (*11*). A high incidence of Coxsackie B infection is usually accompanied by a low incidence of poliomyelitis, and furthermore, when Coxsackie B and poliovirus infections occur simultaneously in the same area, the peak incidence of Coxsackie infection usually precedes that of poliovirus infection by 1 or 2 months (*12*).

Besides the possibility that it represents the effect of interference, the high incidence of Coxsackie B5 virus infection associated with a low incidence of poliovirus infection can also be explained by certain ecologic and immunological factors. They might be the impact of past infections, resistance acquired through vaccinations, balance of immune and susceptible persons, and the introduction of a new virus into a population. The observations made in Mason City (*1*) as well as those reported here for other parts of Iowa indicate that the residents of that State were highly susceptible to Coxsackie B5 virus infection. Hence, introduction of this virus into the State, under favorable conditions, would result in an epidemic. On the other hand, the population of Iowa was relatively resistant to poliomyelitis as a result of past infections, and their immune status had been reinforced by the recent use of the Salk vaccine.

A review of the Iowa data for the past 3 years shows that when the Salk vaccine was first introduced in 1954 there were 1,445 reported cases of poliomyelitis, 525 (36.3 percent) of which were paralytic. In 1955, the number dropped to 556, with 114, or 20.5 percent, paralytic. During the following year there were 580 cases, but only about 8 percent were paralytic. This decline in incidence and in the ratio of paralytic cases coincides with

the introduction and continued use of the Salk vaccine.

As for age distribution of those infected with the B5 virus, rates of infection were approximately the same for persons under 20 years of age in both ill and well categories. These rates were significantly higher than those of the older group. This difference in age-specific incidence indicates that the older population was more resistant to Coxsackie B5 virus infection, possibly because of infection some years ago with an agent similar or antigenically related to Coxsackie virus B5. The uniform infection rates among persons in younger age groups further suggest that Coxsackie virus B5 probably had not been active in Iowa during recent years.

Summary

During the summer of 1956 Coxsackie virus was found widely distributed in several midwestern States. Infections due to this virus appeared in epidemic proportions in several localities in Iowa; about 53 percent of the stools examined yielded Coxsackie virus B5, while about 2 percent contained polioviruses. The frequency of isolating the B5 virus in Kansas, Missouri, and Nebraska ranged from 8 to 12 percent. In Arkansas, none of 104 individuals examined yielded Coxsackie viruses, while 36 percent had polioviruses. One strain of Coxsackie virus B5 was isolated from 15 individuals examined in Oklahoma.

Studies in Iowa also showed that the seasonal incidence of Coxsackie B5 infection was similar to that of poliomyelitis. The frequency of detecting this virus was higher among persons under 20 years than in those over 20 years of age.

REFERENCES

(*1*) Rubin, H., Lehan, P. H., Doto, I. L., Chin, T. D. Y., Heeren, R. H., Johnson, O., Wenner, H. A., and Furcolow, M. L.: Epidemic infection with Coxsackie virus group B, type 5. 1. Clinical and epidemiologic aspects. New Eng. J. Med. 258: 255–263 (1958).

(*2*) Wenner, H. A., and Miller, C. A.: Comparison of methods for recovering poliomyelitis viruses from human sources. Proc. Soc. Exper. Biol. & Med. 86: 11–15 (1954).

(*3*) Youngner, J. S.: Monolayer tissue cultures. I. Preparation and standardization of suspensions of trypsin-dispersed monkey kidney cells. Proc. Soc. Exper. Biol. & Med. 85: 202–205 (1954).

(*4*) Wenner, H. A., Miller, C. A., Kamitsuka, P., and Wilson, J. C.: Preparation and standardization of antiserums prepared with the three known types of poliomyelitis viruses. Am. J. Hyg. 59: 221–235 (1954).

(*5*) Dalldorf, G., and Sickles, G. M.: An unidentified, filterable agent isolated from the feces of children with paralysis. Science 108: 61–62 (1948).

(*6*) Steigman, A. J.: Discussion: Part 1. *In* Viruses in search of disease. Ann. New York Acad. Sc. 67: 249–250 (1957).

(*7*) Syverton, J. T., McLean, D. M., Martins da Silva, M., Doany, H. B., Cooney, M., Kleinman, H., and Bauer, H.: Outbreak of aseptic meningitis caused by Coxsackie B5 virus. J. A. M. A. 164: 2015–2019 (1957).

(*8*) Dalldorf, G.: Sparing effect of Coxsackie virus infection on experimental poliomyelitis. J. Exper. Med. 94: 65–71 (1951).

(*9*) Sulkin, S. E., Wallis, C., and Murphy, T. P., Jr.: Mixed infections with Coxsackie and Lansing poliomyelitis virus in mice. Proc. Soc. Exper. Biol. & Med. 84: 184–189 (1953).

(*10*) Le Bouvier, G. L.: Interference and cell protection by poliomyelitis virus in tissue culture. Nature 174: 649–650 (1954).

(*11*) Dalldorf, G.: The Coxsackie viruses. Ann. Rev. Microbiol. 9: 277–296 (1955).

(*12*) Johnsson, T.: Occurrence of Coxsackie virus infections in an epidemic of poliomyelitis. Arch. ges. Virusforsch. 6: 216–232 (1955).

Correction

In Home Safety Activities, *Public Health Reports*, May 1958, page 455, change "National Health Council" to "National Safety Council."