The New England
Journal of Medicine

Volume 277 NOVEMBER 9, 1967 Number 19

HUMAN INFECTION WITH THE VIRUS OF VESICULAR STOMATITIS DURING AN EPIZOOTIC*

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IN the summer of 1965 an epizootic of vesicular stomatitis, primarily in cattle, occurred in northwest New Mexico and southwest Colorado. The disease was confirmed as the Indiana type by serologic examination of affected cattle and by isolation of the virus from vesicular lesions of cattle by Dr. E. Jenney, of the National Animal Disease Laboratory, Ames, Iowa.

According to preliminary reports obtained from the Animal Health Division, United States Department of Agriculture, the epizootic began in early July in the contiguous counties of Rio Arriba (New Mexico) and La Plata (Colorado) (Fig. 1). The disease spread in an erratic fashion as the summer progressed. About 300 animals were affected in each state by September.

In late July the veterinarians investigating the epizootic suspected that the infection was also occurring among owners and handlers of infected cattle. Stimulated by these reports and at the request of the State Health Departments concerned, the National Communicable Disease Center began field investigations in Colorado and New Mexico.

The results of the epidemiologic and laboratory investigations of the involvement of human beings in the affected areas are reported in this paper. They include the first extensive laboratory documentation of a human outbreak of vesicular-stomatitis infection in association with a natural epizootic.

MATERIALS AND METHODS

Collection of Specimens

During August, 1965, 41 persons in the epizootic area, all of whom had some degree of contact with infected animals, were interviewed, and specimens were obtained for viral isolation and serologic studies. Acute-phase and convalescent-phase serum samples were collected in all suspect cases. Throat swabs were obtained from patients with recent oral or pharyngeal lesions and placed in tryptose phosphate broth with 0.5 per cent gelatin. All specimens were stored at −60°C in a mechanical freezer until shipped to the Communicable Disease Center on dry ice.

Viral-Isolation Attempts

Newborn mice, BS-C-1 (a continuous line of green monkey-kidney tissue culture), and PK_{15} (a continuous line of porcine-kidney tissue culture) were used for attempts at viral isolation.

Each serum and throat-swab specimen was inoculated intracerebrally into 8 newborn mice, 0.02 ml per mouse, and the mice were observed for fourteen days for signs of illness or death. Any mouse with signs of illness was killed; the brain was harvested and passed to another litter of suckling mice. The passage mice were again observed for fourteen days.

The BS-C-1 and PK_{15} tissue-culture systems were examined daily for nine and seven days, respectively, for evidence of cytopathic effect. Tissue-culture material that gave no evidence of cytopathic effect was not passed.

Serologic Technics

The viral strains employed as antigens included Indiana and New Jersey prototypes supplied by Dr. R. P. Hanson, of the Department of Veterinary Science, University of Wisconsin, an Indiana isolate from cattle of the current outbreak supplied by Dr. Jenney, of the United States Department of Agriculture National Animal Disease Laboratory in Ames, Iowa, and a Communicable Disease Center aedes species (?dorsalis) mosquito Indiana isolate from the same New Mexico epidemic area.[1]

Complement fixation tests were performed ac-

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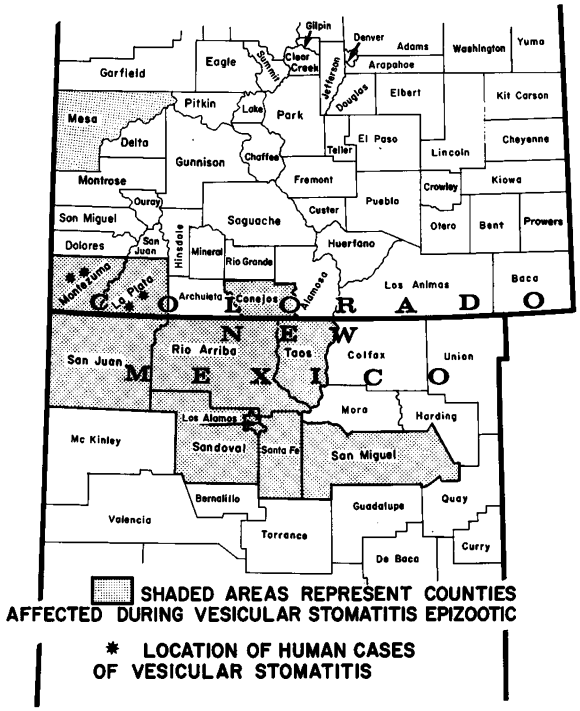

FIGURE 1. *Distribution of Vesicular Stomatitis in Colorado and New Mexico in 1965.*

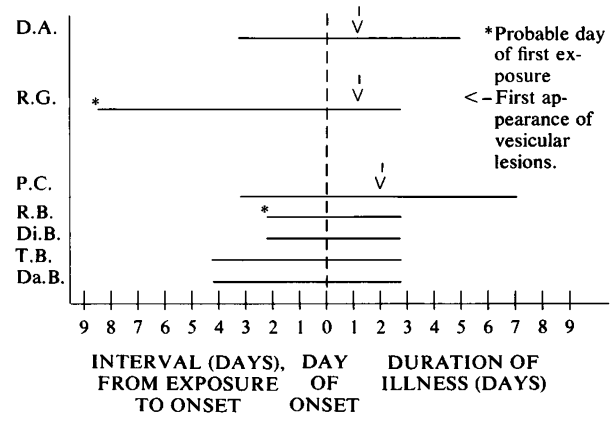

FIGURE 2. *Incubation Period and Duration of Illness in 7 Patients with Vesicular Stomatitis (Indiana Strain) in Colorado and New Mexico, According to Number of Days (1965).*

cording to the standard methods of the Communicable Disease Center.[2] Antigens were prepared from infected mouse brain by means of two methods[3]: the sucrose-acetone extraction technic of Clarke and Casals[4]; and a crude extraction procedure consisting of a 10 to 13 per cent suspension of brain in veronal-buffered diluent (at pH 7.3), centrifugation of the suspension at 2500 rpm for ten minutes and collection of the supernate.

Neutralization tests were performed with the use of the constant serum-virus dilution technic.[5] The virus-serum mixtures were incubated at 37°C for two hours and inoculated intracerebrally into three-week-old mice. Virus for the neutralization tests was prepared from infected suckling-mouse brains harvested from low passages of the Communicable Disease Center aedes mosquito Indiana isolate and prototype.

RESULTS

Clinical Picture and Epidemiologic Data

Eight patients with serologic evidence of infection with the virus of vesicular stomatitis were identified. All 8 had lived or worked on ranches where the Indiana serotype had been serologically confirmed in cattle. These premises were located in 2 Colorado counties, Montezuma and La Plata (Fig. 1). All 8 patients, except 1 whose history was unclear, had close contact with infected animals. Seven of the 8 reported illnesses beginning two to eight days after initial contact with infected animals. The individual illnesses lasted for two to seven days (Fig. 2).

Symptoms were fever, general malaise, myalgia, nausea, vomiting and pharyngitis (Table 1). Within twenty-four to forty-eight hours of onset, 2 patients had vesicular lesions on the gums and the buccal and pharyngeal mucosa, and another had a herpes-like lip lesion. One patient, a thirty-four-year-old man, experienced a 9.1-kg (20-pound) weight loss over a period of three weeks, thereby demonstrating the potential severity of the illness. In this patient pharyngeal vesicular lesions developed within twenty-four hours of the onset of the illness, and buccal submucosal lymphoid hyperplasia was noted on the twentieth day.

Five of the 8 patients were members of 1 family. The father (H.B.) had no history of recent illness whereas all the others with serologically proved cases were ill. However, the father consented to donate only a single specimen, and thus it was not possible to determine whether his titer might have been from a past or a recent infection. The positive complement-fixation results suggested recent infection. Two siblings in this family remained asymptomatic. A serologic specimen was obtained on 1 of these and was negative for vesicular-stomatitis antibody.

In conjunction with the initial epidemiologic investigations, an ecologic survey was conducted. Mosquitos and ectoparasites were collected in the Abiquiu, Taos and Aztec regions of New Mexico. From these collections, 1 isolate of Indiana virus was obtained from an aedes-mosquito pool, the first isolation of this virus from a naturally infected mosquito.[1] In addition, 127 vertebrates, primarily small rodents, were collected from 2 sites in north-central New Mexico. No virus was isolated from the vertebrates or ectoparasites collected from them.[6]

Viral Isolation Tests

No viral strains were isolated from 52 specimens. These included 28 throat swabs and 24 serums collected primarily from persons who had recently had

TABLE 1. *Summary of Symptomatology among Patients with Serologic Evidence of Vesicular Stomatitis (Colorado, 1965).*

PATIENT	AGE	SEX	CHILLS	FEVER	MYALGIA	WEAKNESS	ANOREXIA	HEADACHE	PHARYNGITIS	NAUSEA OR VOMITING (OR BOTH)	OTHER SIGNS
	yr										
D.A.	34	M		X	X	X			X		Temperature of 103°F.; pharyngeal vesicular lesions within 24 hr; buccal lymphoid hyperplasia noted after 20 days; 9.1-kg weight loss over 3 wk.
R.G.	28	M		X	X		X			X	Vesicular lesions on gums & buccal mucosa within 24 hr.
P.C.	42	M		X	X	X	X			X	Temperature of 101°F; marked weakness; herpetic lesion on lower lip within 48 hr.
R.B.*	28	F	X	X	X			X	X		Cervical lymphadenopathy; history of contact with infected animals unclear.
Di.B*	11	M		X				X	X	X	
T.B.*	10	M		X				X	X	X	
Da.B.*	7	M		X				X	X	X	
H.B.†	31	M									

*Members of single family unit.

†Asymptomatic.

infections, but who were not ill at the time of collection. Suckling mice were inoculated with all specimens. In addition, specimens from 18 of the patients were inoculated into BS-C-1, and from 23 patients, into PK_{15} cells. Most of these specimens were throat swabs.

Serology

The results of serologic tests on patients evidencing antibody to Indiana virus are summarized in Table 2.

Initially, results of complement-fixation tests with patients' serum utilizing sucrose-acetone-prepared antigens of standard strains of virus were negative or yielded low antibody titers. Subsequently, sucrose-acetone-extracted antigens, prepared from both the cattle and the mosquito isolates from the area of the epidemic, were used to test the same serums, with similar results. Because of the possibility that the extraction procedure was damaging the antigenicity of the virus, the crude type of complement-fixation antigen prepared from the aedes isolate was utilized. As shown in Table 2, much higher titers were obtained with the crude antigen. Several human serums were then tested in crosshatch or box complement-fixation titrations with each of the antigens (Table 2). In these tests, serum titers were higher with the crude antigen than with the sucrose-acetone-extracted antigen. Unfortunately, the quantity of serum was insufficient for the box titrations to be performed with every specimen. When both the crude and sucrose-acetone antigens were tested by box tests with known hyperimmune homologous mouse ascitic fluids, the results obtained were identical, indicating that the antigens were equally reactive with laboratory-prepared animal serums.

The increased sensitivity of the crude vesicular-stomatitis antigen with human serums prompted an investigation of similar comparisons with naturally immune animal serums. Dr. Jenney kindly supplied several bovine and equine serum specimens that had been collected during the same epidemic and shown by him to be positive for Indiana antibody. The results are summarized in Table 3. When tested by us, the animal serums confirmed the earlier results in a more dramatic fashion.

Laboratory presumptive or confirmed evidence of recent infection was documented in 8 patients. In the 2 (R.G. and P.C.) from whom early acute-phase specimens were obtained a rise in the neutralization index of 2.5 and 2.4 $logs_{10}$, respectively, was demonstrated between the acute-phase and early convalescent-phase serums. These results confirmed infection with homologous virus. In 5 of the other 6 patients the initial serum was drawn at least eleven days after the onset of illness; in these, peak neutralization-test and complement-fixation antibody levels were observed in the initial specimens. Subsequent specimens from all but 1 of these patients, when tested simultaneously with the earlier specimens, revealed falling neutralization-test and complement-fixation titers — strong presumptive evidence of recent infection with the Indiana virus.

TABLE 2. *Serologic Data on Patients with Vesicular Stomatitis, Indiana Strain, According to Date of Onset (Colorado, 1965).*

PATIENT	AGE	SEX	DATE OF ONSET	INTERVAL FROM ONSET TO SPECIMEN	NEUTRALIZATION INDEXES*	COMPLEMENT FIXATION†	
						CRUDE ANTIGEN	SUCROSE-ACETONE ANTIGEN
	yr.			*days*			
D.A.	34	M	7/23/65	20	3.4	64	<8
				110	3.1	8§	<8
				264	1.9	<8	<8
R.G.	28	M	8/5/65	6	1.4‡	<8	<8
				29	3.8‡	16	<8
				97	3.0	<8	<8
				251	2.8	<8	<8
P.C.	42	M	8/6/65	7	0.4‡	<8	<8
				95	2.9	16	<8
				250	2.6	8	<8
R.B.	28	F	8/7/65	12	3.8	128§	32
				94	3.7	32§	16
				289	3.0	16	16
Di.B.	11	M	8/7/65	12	3.9	32§	<8
				94	3.7	8§	<8
				289	3.3	<8	<8
T.B.	10	M	8/8/65	11	3.9	128	32
				93	3.0	16	8
				288	2.9	8	8
Da.B.	7	M	8/8/65	11	3.9	32§	8
				93	3.1	8	8
				288	3.2	<8	<8
H.B.¶	31	M			3.0	8	<8

*Indexes expressed as $\log_{10}$ of protection when virus-serum mixtures inoculated intracerebrally into weanling mice.

†Crude antigen prepared from aedes isolate – suckling-mouse brain harvested, centrifuged at 1500 RPM for 10 min. & supernatant taken as undiluted antigen (antigen used at 1:8 or 1:10 dilutions in barbital-buffered diluent).

‡Test performed at different date – not repeated because of insufficient serum.

§Serum tested by box complement-fixation test with crude & sucrose-acetone antigens.

¶Not ill.

DISCUSSION

Vesicular stomatitis is a viral disease primarily affecting livestock. Two serotypes of the virus, Indiana and New Jersey, have been described. More recently, a third agent, Cocal virus, has been isolated and has been shown to be related to, but different from, the Indiana virus.[7] Although Jonkers et al.[8] have remarked on the resemblance between Cocal-virus epizootics in small rodents and vesicular-stomatitis epizootics in livestock, thus far no known livestock diseases have been attributed to the Cocal virus.[7]

TABLE 3. *Complement-Fixation Tests on Convalescent-Phase Serums* of Animals with Vesicular Stomatitis (Indiana Strain, 1965).*

TEST NO.	ANIMAL	COMPLEMENT FIXATION	
		CRUDE ANTIGEN†	SUCROSE-ACETONE ANTIGEN
1	Bovine	32	<8
2	Bovine	128	<8
3	Bovine	64	<8
4	Bovine	32	<8
5	Bovine	64	<8
6	Equine	≥1024	≥1024
7	Bovine	256	64
8	Bovine	512	32
9	Bovine	128	<8
10	Bovine	128	<8
11	Equine	128	16

*All bled in July, 1965.

†Titers represent reciprocal of maximum serum dilution giving +++ to ++++ fixation of complement at optional dilution when run in box complement-fixation test.

In animals vesicular stomatitis is characterized by the development of vesicles on the oral mucosa, particularly that of the tongue.[9] Lesions may also occur on the udders of cows and mares,[10] and linear necrotic lesions have been observed on the corium and heels of horses and cattle.[9] The disease in cattle somewhat resembles foot-and-mouth disease; however, it produces a much less severe clinical illness. Herd attack rates vary between 5 and 75 per cent, but case fatality rates are usually under 5 per cent.[10-12] The higher attack rates are usually seen in dairy herds, in which contaminated milking cups appear to be a factor in mechanical transmission.[10,13]

The first report in the literature of infection with vesicular stomatitis in human beings was in 1917, when Burton[14] stated that he and an assistant had contracted "stomatitis contagiosa," an illness similar if not identical to vesicular stomatitis. Heiny,[15] reporting an outbreak in Colorado in 1944, described human cases based upon a history of contact and clinical findings. In the 1949 Wisconsin epizootic[12] 1 veterinarian and several herd owners were believed to be infected. No serologic studies were made, and, therefore, no direct proof was obtained that the

reported human illnesses were in fact vesicular stomatitis.

The New Jersey serotype has frequently been implicated as a cause of human illness since the publication of the report by Hanson and his colleagues in 1950.[16] With 2 exceptions,[11,13] these illnesses were acquired in the laboratory. In 1 report a thirty-five-year-old rancher who was exposed to sick horses and cattle contracted an influenza-like illness, with tonsillitis, fever and body pains lasting for six days. Blood specimens showed a diagnostic rise in vesicular-stomatitis neutralizing antibodies. The other report describes 4 men, all exposed to cattle infected with the New Jersey serotype, in whom mild influenza-like illnesses lasting for two or three days developed. Single blood specimens were positive by complement-fixation titer (3 cases) or neutralization test (1 case) to the New Jersey serotype. Serologic surveys, such as those by Hanson and Karstad[17] in southeast Georgia, have also shown that New Jersey antibodies were present in 25 per cent of certain populations.

Report of human disease due to the Indiana serotype are rare. In 1958 Patterson et al[18] noted incubation periods of illness of two, five and eighteen days in laboratory workers after a first exposure to the virus. The clinical symptoms of many laboratory workers exposed to both the Indiana and the New Jersey strains were described as a mild to severe influenza-like illness. In 1964, during an epizootic of vesicular stomatitis due to the Indiana strain, there was a report of serologic evidence of infection based upon examination of single serum specimens of 6 people exposed to affected animals. No clinical data were given.[10] Because paired serum specimens were not tested, the observed serologic results do not necessarily rule out an earlier infection not related to the current illness. Recently, Johnson and his colleagues[19] described serologic and clinical studies of a single laboratory infection after accidental inoculation with the Indiana virus. The illness consisted of fever with generalized myalgia and nausea. All symptoms lasted for three days. Complement-fixation titer and neutralization-test antibody rose early and persisted for several months, as discussed below.

Serologic surveys have been conducted among high-risk groups exposed to the Indiana virus in either the laboratory or the field. Patterson et al.[18] noted that 95 per cent of laboratory workers and animal handlers assigned to vesicular-stomatitis projects over a seven-year period had antibody to the virus – 15 per cent to the Indiana strain alone, 40 per cent to the New Jersey strain alone, and 40 per cent to both serotypes, and in 70 per cent of trainees assigned to such projects for two-week periods positive titers developed – 38 per cent to the Indiana serotype alone, 24 per cent to the New Jersey strain alone, and 8 per cent to both strains. Of the persons studied, 57 per cent with serologic conversion to one or both of the strains had clinical illness. Shelokov and his associates,[20] in 1961, reported a study of 491 serum specimens from long-term residents of a community near a tropical rain forest in Panama. Twenty-seven per cent had neutralizing antibody, including approximately 10 per cent under the age ten and 35 per cent above the age of thirty. Of 130 neutralizing serums, 28 (22 per cent) were reactive to the Indiana antigen on complement fixation. Prevalence of neutralization-test antibody ranged from 0 per cent in urban to 35 per cent in forested areas.

The current studies document naturally acquired human vesicular stomatitis, confirming several features of the illness noted in the reported laboratory-acquired infections. The acute, self-limited influenza-like illness, with fever, myalgia and malaise and a short incubation period, was typical, showing that naturally acquired infection is similar to that acquired accidentally in the laboratory.

The association of all serologically proved cases with sick cattle and the onset of illness shortly after initial contact point to direct contact as the most likely means of human infection. Arthropod transmission does not appear to be implicated in these cases, although an isolation of virus from an aedes mosquito[1] suggests that transmission by arthropod vectors is possible.

The reason for the increased sensitivity of the crude compared with the sucrose-acetone-extracted antigen is not clear. The extracted antigen reacted as well as the crude with laboratory-prepared serums. Although detailed studies were not carried out, the lack of sensitivity of the sucrose-acetone antigen was demonstrated with at least 2 other sucrose-acetone preparations. It is known that there are at least 4 antigenic components to vesicular stomatitis,[21] and it is possible that the extraction technic alters or removes some component. Thus, the reaction of immune serum from a human being or animal subjected to 1 virus challenge may be different from reaction of mouse hyperimmune ascitic fluids produced by multiple injections of antigen. Further studies with other strains of the Indiana as well as the New Jersey strain and other members of the "Stomatoviridae"[22] are needed to evaluate the ultimate significance of these results.

The earliest complement-fixation antibodies were found on the eleventh day although the high titer on that day in the patient (T.B.) indicated that antibody probably was present considerably earlier. The antibody peaked in the first month, but persisted at a 1:8 or 1:16 level in 3 patients for nine or ten months.

The present data on serologic patterns in several naturally infected patients are similar to those reported by Johnson[18] for a single case, with only slight differences. Johnson noted early onset of both neutralization-test and complement-fixation antibody (approximately on the tenth day), with early disappearance of complement-fixation antibody (absent by the hundred and fiftieth day) and persistence of

neutralization-test antibody (over a one-year period). Although the complement-fixation titer was transient in most of our cases, it did persist in 3 patients for at least nine months at low level. Also, neutralization-test titers declined in several of the cases and then persisted at a lower level; in Johnson's report, the antibody persisted at a high level without drop-off.

SUMMARY

The first laboratory-documented outbreak of naturally acquired human vesicular stomatitis (Indiana strain) occurred in 1965 in Colorado in conjunction with an epizootic in Colorado and New Mexico. Important features included close contact of the patients with infected cattle and an acute influenza-like illness of short duration. Serologic studies showed persistent neutralizing-antibody and transient complement-fixation-antibody titers, and a relative insensitivity of sucrose-acetone-extracted antigen as a diagnostic reagent in comparison with crude antigen.

We are indebted to Dr. H. Gordon Doran of the New Mexico Department of Public Health, Dr. C. S. Mollohan of the Colorado Department of Public Health, Dr. J. G. Hill of the Animal Health Division, United States Department of Agriculture, New Mexico, Dr. J. A. Acree of the Animal Health Division, United States Department of Agriculture, Colorado, Dr. Alexander D. Langmuir, Epidemiology Program, and Drs. T. H. Work, R. W. Chamberlain and P. H. Coleman and other members of the Arbovirus Infections Unit, Laboratory Program, National Communicable Disease Center, for assistance and support during this study.

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