Epizootic of Western Encephalomyelitis Virus Infection in Equines in Alberta in 1965

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SUMMARY

The epizootic of equine encephalomyelitis in 1965 in Alberta was proved to be due to Western Encephalomyelitis virus infection by serological findings and virus isolations.

Sixty-three horses of 88 tested, showed a diagnostic rise of CF antibodies to Western Encephalomyelitis virus.

Western Encephalomyelitis virus was isolated from 5 brains of horses. Homologous antibodies were shown in 3 of these animals, the only ones from which blood specimens were received.

For the first time virological evidence is given that Western Encephalomyelitis virus infection in horses is found in more areas of Alberta and in regions situated further North than those previously suspected.

INTRODUCTION

In the summer of 1965 an epizootic of Western Encephalomyelitis occurred in Alberta, with the highest incidence from the second week of August until the end of the second week of September. Thereafter it declined sharply when the Province experienced unusual freezing temperatures and an abundant snowfall that remained on the ground for several days. The appearance of cold weather and its persistence for several weeks in early fall may have contributed greatly to the abrupt ending of the epizootic.

Since the Provincial Laboratory of Public Health contains the only well established Arbovirus Unit of the Province, it was called on by the Laboratory Branch of the Alberta Veterinary Services Division to carry out examinations of sera from sick horses and of brains of horses suspected to have died of Western Encephalomyelitis infection.

TABLE I. Number of Clinical Cases of Horses with Encephalitis, Reported Weekly to the Laboratory Branch of the Provincial Veterinary Services of Alberta, in 1965.

	1st week	2nd week	3rd week	4th week	5th week
JUNE			1		2
JULY					1
AUGUST	7	21	84	109	22
SEPTEMBER	21	30	8	6	1
OCTOBER		6	2	1	

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MATERIALS AND METHODS

A total of 322 horses, clinically ill with encephalomyelitis, were weekly reported to the laboratory branch of the provincial veterinary services from the 3rd week of June until the 4th week of October (Table 1). Paired, acute and convalescent sera were received from 88 horses all after the first week of August. Single sera were received from 133 horses and no specimens from the remaining 101. All sera were tested for the presence of complement fixing antibodies to Western (WE), Eastern (EE) and St. Louis (SLE) Encephalitis viruses. The brains of 35 horses were also received.

A. VIRUS ISOLATION ATTEMPTS

Isolation of viruses was attempted only from the brains of the dead horses. The procedures used were those described by Lennette and Schmidt. (2)

Twenty percent suspensions of different sections of brain (cerebrum, thalamus, midbrain and medulla oblongata) were prepared in 25% normal rabbit serum-buffered water, pH 7.6-7.8, containing antibiotics, and centrifuged at 10,000 r.p.m. for one hour in a refrigerated centrifuge. The supernatant fluids were decanted into sterile ¼ oz. glass bottles. Part of each aliquot was used for virus isolation and the remainder was stored at -70°C for later isolation attempts. Each specimen was inoculated into: (a) swiss mice, 1-2 days old, by intracerebral and intraperitoneal routes; (b) the amniotic cavity of 8-day-old embryonated hens' eggs; (c) four tissue culture tubes of chick embryo fibroblasts.

Mice were inoculated as previously described by Morgante *et al* (5). Examination of all mice was made for 14 consecutive days. Sick mice were selected for passage. Dead mice were discarded. No blind passages were made. Reisolation of the strains from the original brain suspension was attempted in all instances and consistently obtained. Serological identification of the isolates was accomplished by neutralization tests in suckling mice. A crude 20% mouse brain suspension was used as antigen. Hyperimmune mouse ascitic fluid was used as immune serum, prepared with WE virus, Fleming strain (T.F.), obtained from Dr. P. H. Coleman, Communicable Disease Center, Atlanta, Georgia. The immune serum was inactivated at 56°C for 30 minutes prior to use. Constant serum-varying virus dilution mixtures, after one hour incubation at 37°C, were inoculated intraperitoneally into a litter of eight swiss mice, 2-4 days old.

Inoculation of 10-11-day-old hens' embryonated eggs was performed by the amniotic route. Six eggs were used for each specimen. The eggs were candled daily for seven days, after which period they were discarded if considered negative. Embryos

TABLE II. Complement Fixing Antibodies to WE Virus in Paired Sera of Horses Ill with Encephalomyelitis

	No. Paired Sera Rec'd.	No. with Diagnostic Rise	No. with no Diagnostic Rise	No. Negative
August, Week				
1st	3	2	0	1
2nd	9	7	1	1
3rd	17	14	1	2
4th	26	16	8	2
5th	13	9	2	2
September, Week				
1st	6	5	0	1
2nd	12	8	3	1
3rd	1	1	0	0
4th	0			
5th	0			
October Week				
1st	0			
2nd	1	1		
3rd-5th	0			
Total No.	88	63	15	10

TABLE III. Titers of Complement Fixing Antibodies to WE Virus in Paired Sera of Ill Horses in which no Diagnostic Rise was Demonstrated

	CF Antibody Titers, Acute Serum: Convalescent Serum						
	8:8*	8:16	16:16	16:32	32:32	32:64	64:128
No. of paired sera.....	2	1	3	2	3	2	2

*The serum reciprocal dilution

dying after 24 hours from inoculation were harvested and passaged in another set of embryonated eggs and thereafter checked for infectivity by intracerebral inoculation in suckling mice. Inoculation in mice represented the reference host system to control all the specimens that showed cytopathic effect (CPE) in tissue culture.

B. SEROLOGICAL INVESTIGATION

1. *Complement Fixation (CF) Test*: All sera were tested by the Standardized Diagnostic Complement Fixation Method, the macrotechnique as described by Casey (1). WE, EE and SLE viruses were used as antigens.

2. *Neutralization Test (NT)*: When blood serum was available, neutralizing antibodies were determined in the animals from the brains of which WE virus was isolated. The NT results were expressed in terms of Neutralization Index. The LD_{50} end points for this purpose were calculated by the Reed-Muench formula (2).

C. INTERPRETATION OF RESULTS

When WE virus was isolated from the submitted brains, it was accepted as the etiological agent of the disease. In those instances in which homologous CF and NT antibodies could be demonstrated in addition to virus isolation, this was considered as further support of the diagnosis of WE.

Where only serology was performed, a horse was accepted as a "proven positive" case if a fourfold or greater increase of CF antibody levels was demonstrated between the acute and convalescent blood sera. When only a single serum sample was submitted, CF antibody titers of 1:32 or higher were considered as presumptive evidence of WE virus disease.

TABLE IV. Complement Fixing Antibody Titers to WE Virus in Single Sera of Horses Ill with Encephalomyelitis

		CF Antibody Titers							
	No. Single Sera Rec'd.	<8	8*	16	32	64	128	256	512
August, Week									
1st.....	1	1							
2nd.....	9	6	2		1				
3rd.....	43	9	2	3	11	10	7	1	
4th.....	43	10	3	8	14	4	4		
5th.....	8	1	2			3		1	1
September, Week									
1st.....	17	4	2	3	3	2	2		1
2nd.....	6	1			2	3			
3rd.....	2	1					1		
4th.....	2					1		1	
5th.....	0								
Total No.....	131	33	11	14	31	23	14	3	2
October —	No sera received								
June —	One serum received in the 3rd week : no WE antibodies detected								
July —	One serum received in the 5th week: no WE antibodies detected								

*The serum reciprocal dilution
0 indicates no serum received

TABLE V. WE Virus Findings of Five Horses which Died with Encephalomyelitis

Lab No. and Age of Animal	Location	Onset of Disease	Date of Death	Specimens Received	Date Rec'd.	Virus Isolated	Antibody Titer to WE Virus CF	Antibody Titer to WE Virus Log TD_{50} NT Index
Ho-1 10 years.....	Edmonton	VIII.14	VIII.17	Brain	VIII.18	WE	16	1.7
				Blood				
HO-2 6 years, (F).....	Beiseker	VIII.16	VIII.17	Brain	VIII.19	WE		
HO-3.....	Edmonton	VIII.17	VIII.19	Brain	VIII.19	WE		
				Blood	VIII.18		32	2.0
HO-4.....	Edmonton	VIII.21	VIII.23	Brain	VIII.30	WE		
				Blood	VIII.23		32	NSQ
HO-5 24 years, (F)....	Rimbey	IX.8	IX.9	Brain	IX.10	WE		

NSQ indicates not sufficient quantity.

RESULTS

Two hundred and twenty-one horses, clinically ill with encephalomyelitis, were serologically investigated (Table II and III). Sixty-three horses of the 88 from which paired sera were received, showed a diagnostic rise of CF antibodies to WE virus, whereas no antibodies to EE and SLE viruses were detected. Fifteen horses had antibody levels to WE virus varying from 1:8 to 1:128 although a diagnostic rise was not demonstrated (Table III). Three horses, reported to have been vaccinated with WE virus were excluded from these series.

The results of 133 horses from which only one blood specimen was received, are tabulated in Table IV. Ninety-eight of these animals showed antibody levels to WE virus: 25 had titers from 1:8 to 1:16 and 73 had titers from 1:32 to 1:512. No specimen was submitted for virus isolation from any of these animals.

Thirty brains of the 35 received from horses which died of an encephalomyelitis clinically suspected to be due to WE virus, did not yield any virus on isolation attempts. From five brain specimens, WE virus was isolated in mice, hens' embryonated eggs and chick embryo tissue culture. Two of these horses showed both CF and NT in the blood serum, while from a third horse only CF antibodies could be determined since the quantity of serum received was insufficient for further tests (Table V). No blood serum was received from the other two horses.

1. Histopathology

The findings reported include only the five brains from which WE virus was isolated. None showed any gross lesions apart from moderate congestion. Detailed histological examinations of the brain were not made, but blocks were usually taken from any area of the cerebral cortex, cerebellum, and brain stem and stained with hematoxylin-eosin. The findings are listed below:

Cerebrum — The most common finding was infiltration of blood vessel walls and perivascular spaces with lymphocytes, glial cells and a few macrophages. A few areas of liquefaction and gliosis were seen, but in only one case was neuronal degeneration and necrosis with neuronophagia evident. Capillary congestion and many petechial hemorrhages were seen in two brains.

Brain stem — All showed mild to moderate "cuffing" of blood vessels. In two there was severe congestion with hemorrhage into perivascular spaces. Only one had distinct neuronal necrosis with a few neutrophils in the area.

Cerebellum — No lesions were found in four of the five examined. The fifth had mild cuffing of blood vessels.

2. Clinical Findings

Detailed results of clinical examinations seldom accompanied the specimens. They

TABLE VI. Prominent Signs and Symptoms of Forty-Eight Positive Horses

Sign	No. of Cases	% of Total Cases
Incoordination	39	81
Depression	25	52
Ataxia	23	48
Circling	7	15
Facial tremors	5	10
Hyperaesthesia	5	10
Grinding teeth	4	8
Impaired vision	1	2

were obtained only from 48 of the 63 positive horses in which the diagnosis was confirmed by serology or virus isolation (Table VI). We point out that the figures in the Table are only a rough guide because of overlapping symptoms in many of the cases, and lack of their complete recording in others. Incoordination was a feature of the great majority of cases. Many horses were recumbent for two or more days before death. In many others, a "sleepy-looking" horse, difficult to arouse, with a head-down stance, was described. Several of these animals became alert and responded well to stimuli when aroused from their stupor. Excitability and hyperaesthesia were much less common, but this may be due more to the stage of the illness when examined than to the true frequency. They were described by Mitchell *et al.* (4) as very common signs in the early stages of the disease in experimentally infected horses. More than 75% of the horses had increased temperatures (102.5°C. or more) when first examined. There seemed to be no essential difference between the clinical signs described in these cases and those described in the 1938 outbreak in Canada (3).

The incidence of cases in various age groups is shown in Table VII. Age was known in only 41 of the positive cases. Young horses appear to be more susceptible than the older ones.

DISCUSSION

The epizootic of 1965 was shown to be due to WE virus infection by serological findings and isolation of the virus from the brains of the dead animals.

Sixty-three of the 88 horses from which paired sera were received, were proved to be positive by a diagnostic rise of CF antibodies to WE virus (Table II).

Definite proof of acute WE virus infection could not be obtained from the 133 horses which had only one blood specimen examined (Table IV). However, the high antibody levels shown by 73 animals considered as presumptive evidence that these horses also were acutely ill with WE virus infection.

WE virus isolations were obtained from 5 brains out of the 35 tested (Table V). Subsequently it was learned that a number of these brains had been kept on dry ice, without adequate sealing, before shipment to the Laboratory Branch of the Provincial Veterinary Services. Several other factors, however, may have contributed to the low incidence of virus isolations since the brain specimens came from parts of Alberta where adequate shipment and means of transportation were not always available.

Three brains which yielded Western Encephalomyelitis virus came from horses of the Edmonton area. In these three horses homologous antibodies to WE virus were also demonstrated (Table V). It is known that two of these animals, a draft horse and a children's pet, were the only horses on the farm, from which they had never been moved. This is the first time that proof of WE virus infection in the Edmonton area has been recorded, through virus isolation and homologous antibody demonstration in the animals' blood sera. Western Encephalomyelitis was thought to be confined to the southern and east-central re-

TABLE VII. Western Equine Encephalomyelitis, 1965: — Distribution of Cases by Age

	Years of Age							
	0-1	1-2	2-3	3-4	4-5	5-10	10-20	20-30
No. of Horses	6	3	7	4	6	10	3	2

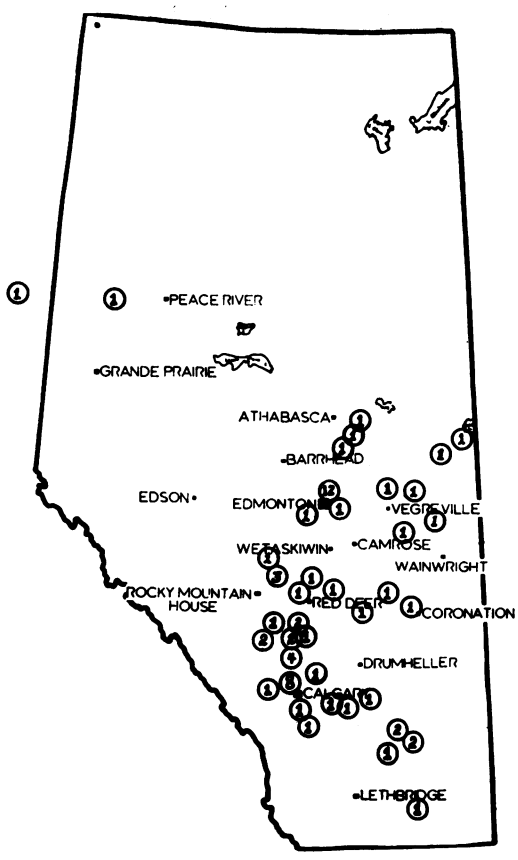

Fig. 1. Geographical distribution of the proven cases of WE virus infection in horses in Alberta, 1965.

gions of Alberta. However, the 63 serologically proven positive horses, which came from areas scattered throughout the province (Fig. 1 — Map), constituted evidence that WE virus infection is found in more areas and in regions situated further north than previously suspected. Proven human cases of WE virus infection were less numerous and limited only to the south of the province (6). The contrast in the geographical distribution between humans and horses may perhaps be attributed to the distribution of the mosquito vectors in Alberta. Until now the only evidence of WE virus infection in arthropods was obtained from mosquitoes collected in the south of the province, where *Culex tarsalis,* the most important human vector so far known, is dominantly confined (7).

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