OCTOBER, 1940 TECH. BULLETIN No. 50

AGRICULTURAL EXPERIMENT STATION

KANSAS STATE COLLEGE OF AGRICULTURE
AND APPLIED SCIENCE

MANHATTAN, KANSAS

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PRINTED BY THE
KANSAS STATE COLLEGE PRESS
MANHATTAN, KANSAS
1940

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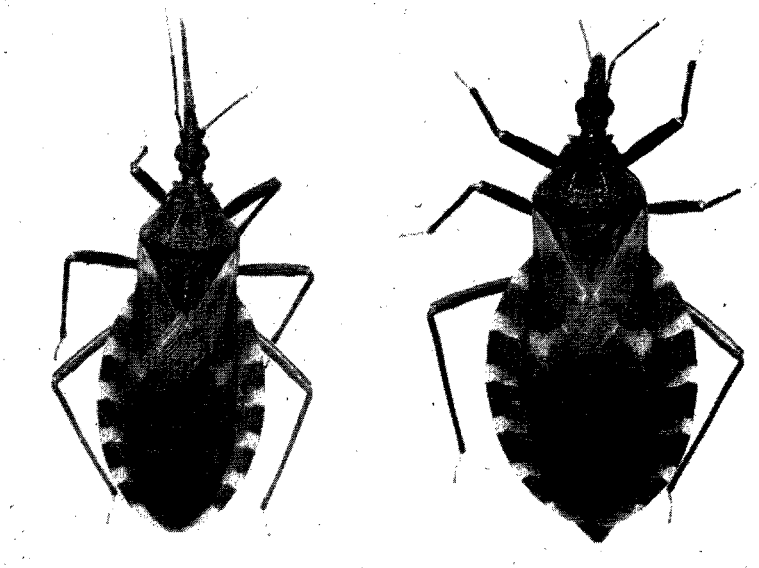

Fig. 1—Male (left) and female (right) of *Triatoma sanguisuga* (*Le Conte*), transmitters in nature of the virus of equine encephalomyelitis. A brief note on the biology of these insects is contained in the appendix.

Equine Encephalomyelitis Virus Isolated From Naturally Infected *Triatoma sanguisuga* LeConte[1]

BY CHARLES H. KITSELMAN AND ALBERT W. GRUNDMANN

The neurotropic virus of equine encephalomyelitis, fatal to guinea pigs, was isolated from a collection of *Triatoma sanguisuga* Lec. obtained from a pasture near Garrison, Kans., in June, 1940. This insect, commonly called "assassin bug" is of the family Reduviidae, and is common throughout Kansas and over much of the region where equine encephalomyelitis has occurred. It is a large, bloodsucking bug and in nature is known to feed upon horses. (See Fig. 1).

Further collections were made and the virus was demonstrated in three of five separate lots taken in pastures in June and July. The area from which the collections were made constitutes several square miles of natural pasture land in which horses are grazing and in which several clinical cases of the disease occurred last season. Two horses that had been pastured in the field in which the first collection was made died from encephalomyelitis in 1939.

Studies indicate that in all respects the virus obtained is identical with the Western strain of equine encephalomyelitis. This paper presents the first known evidence of the virus of equine encephalomyelitis to be found in a bloodsucking insect in nature that is known to feed upon horses and further presents the evidence that this virus is that of equine encephalomyelitis.

After collection, the insects were transported alive to the laboratory where they were ground in a mortar, centrifugalized at high speeds, and passed through a porcelain filter in order to obtain a bacteria-free filtrate. This filtrate was inoculated intracranially into susceptible 300-gram guinea pigs which were kept under observation and temperatures taken morning and night.

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[1]Contribution No. 84 from the Department of Veterinary Medicine and No. 495 from the Department of Entomology.

This work has been financed by a special grant from the Horse and Mule Association of America and by funds from the Kansas Agricultural Experiment Station. The authors wish to thank Dr. Roger C. Smith of the Department of Entomology and Dr. L. M. Roderick of the Department of Veterinary Medicine for valuable technical advice and assistance.

On the first successful attempt, 50 percent of the guinea pigs inoculated died in six days, demonstrating symptoms characteristic of a neurotropic virus. The remaining 50 percent recovered after demonstrating a typical reaction. The brains of the guinea pigs that succumbed were removed and preserved in a buffered-glycerin-saline solution and stored in the refrigerator. A candle filtrate was prepared from this material and inoculated intracranially into a second series of guinea pigs resulting again in a 50-percent mortality, but accompanied by a shortening of the course of the disease to four days. Six serial guinea pig passages were completed before attempts were made to type the virus. Following the second passage, the virus became fixed to cause 100 percent mortality in guinea pigs in four days. The virus, when first isolated, appeared to be of low virulence but built up rapidly by serial passage. The virus also killed consistently following intranasal instillation and foot pad inoculation.

The virus was typed by the following experiments (Table I). Ten day incubated chick embryos were killed by the virus in 18-36 hours. Rabbits proved refractory, showing elevated temperatures with usual recovery. English sparrows, pigeons and white rats proved to be 100 percent susceptible to intracranial inoculation. A bull calf was inoculated intracranially and succumbed to the virus in four and one-half days. A forty-five pound lamb proved to be completely resistant to intracranial inoculation and did not demonstrate any rise in temperature or other abnormal symptoms. A six months old colt succumbed three and one-half days following intracranial inoculation of the virus. The virus was recovered from the brain filtrates of all of the above susceptible animals by guinea pig inoculation.

The tabulation of the typing studies led to the belief that the virus was a strain of equine encephalomyelitis. This belief was further supported by the manifestation of characteristic symptoms in inoculated guinea pigs, namely, creeping paralysis following footpad inoculation, grinding of the teeth and the swimming motion of the front limbs. Salivation was usually present as a symptom.

Two strains of the virus of equine encephalomyelitis are recognized in the United States. The strain localized to the region east of the Appalachian mountains is designated as

the Eastern and that found in the region west of these mountains is designated the Western strain. In only one state, Alabama, have both strains been found.

To check this assumption, a group of guinea pigs[2] solidly immune to the Eastern strain of equine encephalomyelitis were obtained and proved to be 100 percent susceptible to the *Triatoma* virus, dying in four to five days with typical symptoms following intracranial inoculation. (Table II). Another group of guinea pigs solidly immune to the Western strain proved to be 100 percent immune to the same virus. Those animals had been immunized with Eastern and Western Commercial Chick vaccine, respectively. Each had received the prescribed two doses seven days apart followed by a challenge dose of the virus ten days later.

In propagation work and in diagnosis two methods are recognized as standard. One is the detection of the virus through the medium of inoculating a hen's egg containing a 10-day incubated living chick. The virus multiplies many thousand fold in this medium. The second method is one of animal inoculation from guinea pig brain to guinea pig brain. In some instances mice are used as experimental animals.

The cross-immunity studies were repeated with other groups of guinea pigs (Table III) which were solidly immune against either the Eastern or Western strain of equine encephalomyelitis virus, respectively. It was again demonstrated that those guinea pigs immune to the Western virus were immune to the *Triatoma* virus while those guinea pigs immune to Eastern virus were susceptible to the *Triatoma* virus.

The histological findings, following examination of the sectioned brains of animals dying from this virus, showed them to be indistinguishable from those produced in typical cases of Western equine encephalomyelitis. Attempts were made to demonstrate the cell inclusion bodies of distemper in the bladder mucosa and the Negri bodies of rabies were unsuccessful. This, together with the results of the above cross-immunization tests and animal typing studies, confirms the belief that the *Triatoma* virus is identical with Western strain equine encephalomyelitis virus.

[2]The Guinea pigs used in the cross immunity studies were obtained through the courtesy of the Bureau of Animal Industry and the Jen-Sal Laboratories of Kansas City.

The live insects from each group not used for direct isolation of virus were placed in separate cages and fresh susceptible guinea pigs were placed in contact with them to see if the insects could transmit the virus through biting and feeding. In one instance the guinea pig succumbed 28 days after entering the cage and the virus was isolated from the brain. This virus is now in the third serial passage.

Experiments planned to clarify the role of the insect in carrying the virus throughout the year and its relation to outbreaks of equine encephalomyelitis are now in progress.

APPENDIX

TABLE 1

Virus Typing Studies

Animal species used	Number of each used	Percentage of mortality	Percentage of recovery	Observations
10-day incubated chick embryo	10	100	0	18-36 hours
Rabbits	3	33⅓	66⅔	6-7 days
English Sparrows	12	100	0	1-2 days
Pigeons	2	100	0	4-5 days
White Rats	2	100	0	4 days
Calf	1	100	0	4½ days
Lamb	1	0	100	21 days Released
Guinea Pigs	48	91	9	4-5 days
Colt	1	100	0	3½ days

TABLE 2. CROSS IMMUNITY STUDIES

Guinea pigs immune to Eastern strain

Ear Tag		Prelim. temp.	Days 1	2	3	4	5	6	7	8*	9	10	11	12
182	A. M.	103.7	102.0	102.7	106.3	105.8								
	P. M.		102.4	103.9	106.8	Dead								
188	A. M.	103.1	102.8	103.0	105.3	106.4	105.7							
	P. M.		103.7	104.0	106.1	106.6	Dead							
194	A. M.	104.6	103.8	105.9	106.2	99.0								
	P. M.		106.6	104.8	104.8	Dead								
185	A. M.	104.2	103.1	103.6	106.4	106.4								
	P. M.		103.1	104.6	106.6	Dead								
186	A. M.	104.5	102.8	102.6	105.6	106.6	106.0							
	P. M.		102.7	103.2	106.2	106.8	Dead							
Control—no virus injected														
187	A. M.	102.5	102.1	102.1	102.4	102.8	102.8	102.9	103.5	102.8	101.0	101.8	101.2	102.0
	P. M.		102.2	102.0	103.0	103.0	103.2	104.2	105.7	106.0	103.2	103.3	103.6	103.2
183	A. M.	103.2	101.0	101.1	102.4	102.5	102.0	102.5	103.7	103.2	101.4	102.4	101.5	102.5
	P. M.		101.5	102.5	103.2	102.8	102.9	104.4	105.9	107.0	102.6	103.7	103.8	103.5
Control pigs released on the 13th day.														

Guinea pigs immune to Western strain

195	A. M.	103.4	100.2	101.1	102.5	101.7	102.3	103.0	103.3	103.1	102.0	101.7	101.5	101.5
	P. M.		101.9	102.2	103.6	103.2	103.6	104.7	103.3	105.6	104.6	103.9	104.0	103.5
190	A. M.	102.2	102.5	101.6	101.8	102.5	103.3	103.6	103.6	102.3	101.5	102.2	101.7	101.9
	P. M.		102.7	102.9	102.9	103.0	103.4	105.5	105.6	104.8	103.0	104.0	103.8	103.9
191	A. M.	104.6	102.5	101.5	102.4	102.7	103.0	102.6	103.6	101.6	101.8	102.4	103.7	102.7
	P. M.		101.2	102.5	103.5	103.6	103.6	104.3	106.6	106.5	103.8	104.1	103.7	103.9
193	A. M.	103.6	102.6	101.1	102.9	103.1	103.0	103.5	104.0	103.8	103.3	102.1	101.1	102.1
	P. M.		102.2	102.4	104.4	103.2	103.4	104.4	105.9	107.0	104.2	103.9	104.2	104.1
Control—no virus injected														
184	A. M.	104.0	100.9	101.1	101.5	101.4	101.8	102.8	103.3	102.4	102.0	101.0	101.6	101.3
	P. M.		101.0	101.8	102.2	101.6	102.5	103.6	105.2	105.5	102.5	104.2	104.0	103.6
189	A. M.	104.0	101.4	101.4	103.0	103.0	102.4	103.0	103.9	103.7	103.2	102.2	103.9	103.1
	P. M.		101.6	102.0	103.6	103.2	103.1	104.8	105.8	107.3	104.1	104.3	104.0	103.9
196	A. M.	104.1	101.8	101.8	102.5	103.2	103.2	103.5	104.2	103.4	102.9	102.6	103.2	103.1
	P. M.		103.1	103.6	103.4	103.2	102.9	104.0	105.3	104.3	104.4	104.5	104.0	104.1

Control pigs released on the 13th day.
*The temperature in Manhattan on this day was 107 degrees.
Note—All except the control pigs received 0.2 cc. of a 1% guinea pig brain virus filtrate.

Guinea pigs susceptible to Equine Encephalomyelitis

163	A. M.	102.4	103.3	104.4	104.0	100.5
	P. M.		105.4	106.1	105.4	Dead
176	A. M.	102.7	103.0	104.0	104.9	102.0
	P. M.		104.8	104.9	105.0	Dead

Note—These susceptible pigs received 0.2 cc. of a 1% guinea pig brain virus filtrate intracranially.

TABLE III. CROSS IMMUNITY STUDIES No. 2

Guinea pigs immune to Eastern strain

Ear tag No.		Preliminary temperature	Days 1	2	3	4	5	6	7
183	A. M.	103.0	105.7	107.0	105.2	dead			
	P. M.		107.2	107.4	100.1				
187	A. M.	102.2	103.7	104.3	106.8	dead			
	P. M.		106.0	105.1	104.0				
220	A. M.	103.0	104.7	106.4	107.2	dead			
	P. M.		107.8	107.1	103.1				
Control—no virus injected									
226	A. M.	104.2	103.4	104.4	104.0	103.3	103.1	103.9	102.5
	P. M.		106.6	107.3	108.0	104.4	104.0	104.3	103.9
228	A. M.	104.5	104.4	104.3	103.7	103.4	103.7	103.6	104.6
	P. M.		104.8	106.1	105.7	103.5	104.4	104.1	104.2

Guinea pigs immune to Western strain

184	A. M.	102.3	102.7	102.0	101.8	102.3	102.5	103.1	103.1
	P. M.		106.3		106.0	104.0	104.2	104.0	104.2
189	A. M.	103.1	106.5	106.6					
	P. M.		107.5	dead*					
196	A. M.	102.9	105.9	104.7	102.8	101.4	104.6	104.6	104.2
	P. M.		107.4	105.5	104.6	104.4	104.7	104.3	104.0
230	A. M.	104.8	104.9	104.2	104.2	104.1	103.1	105.4	103.4
	P. M.		106.5	105.9	105.0	104.3	104.1	104.5	104.4
240	A. M.	103.5	103.5	103.2	102.9	102.8	102.2	103.1	103.0
	P. M.		105.9	106.4	105.2	103.5	104.1	104.5	104.1
Control—no virus injected									
241	A. M.	103.0	101.5	102.3	dead				
	P. M.		104.4	105.7					
243	A. M.	104.3	103.8	104.8	104.7	104.2	103.4	103.1	104.0
	P. M.		106.0	105.3	106.8	105.0	104.2	104.3	104.5

*—No virus isolated. Note—All except the control pigs received 0.2 cubic centimeters of a one percent guinea brain virus filtrate.

Control—Triatoma virus

244	A. M.	102.7	103.8	104.9	104.8	dead			
	P. M.		107.7	106.8	106.0				

Note: The susceptible guinea pig received 0.2 cubic centimeters of a one percent guinea pig brain virus filtrate intracranially.

History of Equine Encephalomyelitis

The first published reference to equine encephalomyelitis was contained in the writings of Prof. Alfred Large of the New York City Veterinary College, who described the disease among horses on Long Island. Professor Large stated that the disease "has prevailed in epidemic form at various times over a period of 18 to 20 years." At that time the disease was variously known as "staggers," "putrid fever," and paralysis. Professor Large was the first to call the disease "cerebro-spinal meningitis" and for the first time described its symptoms.

The next published reference to work done was by Williams in 1897, in describing an outbreak in the Snake River Valley in Idaho in the winter of 1897. Both stabled horses and those kept in cultivated fields were affected. The climate and altitude forbid the suggestion of mouldy forage and as a precaution Williams checked both feed and water. The symptoms described by Williams are identical with those accepted today.

In 1900 Pearson of the University of Pennsylvania Veterinary College experimented and attempted to produce equine encephalomyelitis artificially by feeding different materials. All feeding trials resulted negatively. Tests were made with mouldy silage which killed the animals but produced symptoms unlike those of a typical case of the disease. He suggested the name "forage poisoning."

Buckley and MacCallum reported on an outbreak of equine encephalomyelitis in Maryland in 1901. Affected animals exhibited symptoms now recognizable as those of the Eastern strain of the disease.

In 1902 and 1903 Butler produced fatal results by feeding mouldy corn but stated that the disease differed markedly from the so-called cerebro-spinal meningitis.

Udall, in 1912, reported an outbreak of equine encephalomyelitis in Kansas and related the disease to the Borna disease of horses in Europe. He based his conclusions on the work of Joeg in Germany, and believed that he discovered inclusion bodies in the nerve cells of the brain and thought them to be Chlamydozoa. "Chlamydozoa are referred to as

pathogenic micro-organisms capable of passing through bacterial filters," Udall stated. "They appear to be more closely associated with the Protozoa than the bacteria. Their development is intranuclear. Others which belong to this group are the causes of vaccinia, trachoma, rabies and perhaps hog cholera," he added. The group mentioned by Udall evidently is that group now called viruses. Udall did not believe the disease was caused by food but thought that it was in the soil and transmitted to the animal through the nose. He correlated the incidence of the disease to weather conditions and to plowing.

Outbreaks of the disease became especially severe in Kansas in 1912, and the state had suffered acute outbreaks in 1891, 1902 and 1906.

In 1930 Myer, Haring and Howitt definitely established the causative agent of the disease to be a filterable virus. From this time onward rapid progress was made toward the solution of the disease. Myer also expressed the belief that the virus was transmitted by an insect vector. This was later borne out by Col. R. A. Kelser (1933) who proved that *Aedes aegypti,* the yellow fever mosquito, could successfully transmit the virus from infected to susceptible guinea pigs. This work was followed in rapid succession by that of Simmons, Reynolds and Cornell (1934), with the successful incrimination of *Aedes albopictus,* and that of Merrill, Lacilaide and Ten Broeck (1934) who succeeded in transmitting both Eastern and Western strains through the agency of *Aedes sollicitans,* the New Jersey Salt Marsh mosquito, and also *Aedes cantator.*

Madsen and Knowlton (1935) added *Aedes nigromaculis* and *A. dorsalis* to the list of transmitters and Ten Broeck and Merrill (1935) and Kelser (1937) added *A. vexsans* and later Kelser (1938), *A. taeniorhynchus.* Davis (1940) has added *A. triseriatus* to this group of experimentally infected mosquitoes.

Further laboratory work with mosquitoes has apparently established the ability of members of the *Aedes* group to transmit the disease while experimental work with members of other groups has consistently proved to be negative. As far as known at this time, only three species of the *Aedes* group are common to Kansas. They are: *A. triseriatus, A. vexans* and *A. nigromaculis,* all of which have been shown ex-

perimentally to be able to transmit the disease under laboratory conditions. The vast majority of the mosquitoes present in this region, however, are of groups other than *Aedes*.

Syverton and Berry (1936) have shown that the Rocky Mountain Spotted Fever tick, *Dermacentor andersoni,* is able to transmit the disease from guinea pigs to ground squirrels experimentally and further suggest that the gopher, *Citellus richardsoni* may be the natural host of the virus because of its extreme susceptibility.

A Note on the Biology of *Triatoma sanguisuga (LeConte.)*

Triatoma sanguisuga (Le Conte) is one of the larger cone nosed or assassin bugs of the family Reduviidae. It is closely related to the so-called "kissing bug" or "masked bed bug hunter." This species is tropical and sub-tropical in distribution but its range extends northward throughout the Great Plains area. Closely related forms occur practically all over the United States, but in no state could any of the forms be regarded as a common or abundant species.

All stages of the bugs live in rodent burrows and nests. They have been most readily taken around Manhattan in the burrows of wild rodents, under stony ledges on hill sides. They feed largely on the blood of vertebrates, and have been reported as attacking other insects also. They overwinter as about half-grown nymphs to adults. The immature forms become adult from early May to August. Eggs are deposited from June to September. There is only one generation a year.

This assassin bug is nocturnal in habits, feeding and flying about chiefly at night. They are attracted to lights during mid-summer evenings and frequently enter homes, seeking especially bed rooms and basements. Persons are sometimes bitten by them.

An excellent, detailed account of the biology of this and other species of assassin bugs has been written by P. A. Readio and published in the Science Bulletin, University of Kansas, Vol. 17, No. 1, 291 pages, 21 plates, December 1, 1927.

—Roger C. Smith.

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