Report of an epizootic of Encephalomyocarditis virus in Pará, Brazil*

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Beginning in January 1960 a virus which killed infant mice in one or two days following intracerebral or intraperitoneal injection and produced paralysis in adult mice when it did not kill them, was isolated with increasing frequency in the Instituto Agronômico do Norte (IAN) and Utinga study areas from sentinel mice, wild rodents, and mosquitoes and from one *Marmosa opossum* at Utinga. The strain selected as prototype was AN 17289, isolated from liver of a spiny rat, *Proechimys guyannensis oris*.

Brain antigen of AN 17289 reacted with homologous serum in complement fixation testing but not with hyperimmune sera of arborviruses known in Belém, nor with mouse Encephalomyelitis hyperimmune serum. It was not inactivated by sodium desoxycholate, or by ether after overnight incubation at 4ºC. Embryonated eggs, seven days old, were inoculated by the yolk sac route. Three of four died at 48 hours with hemorrhagic embryos, but embryo material passed to nine day old eggs was not pathogenic for them. Crude and sucrose-acetone extracted mouse brain failed to agglutinate goose cells. Crude brain agglutinated sheep cells in saline diluent at 1:160 dilution of 10% suspension. An HI test on lucite plates was devised using these cells and serum-virus mixture incubated for one hour at 37ºC. Agglutination patterns were much improved when the sheep cells were suspended in

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saline 4.5gm/100cc and the test incubated at 4ºC after addition of cells. Sheep cell agglutinins must be removed from sera by absorption prior to testing. The hemagglutinin of AN 17289 was inhibited by homologous and not by a Guamá antiserum. No work has been done with nonspecific inhibitors and their removal, and it is not known whether this will prove to be a satisfactory technique for antibody testing.

This characterization, together with the clinical appearence of infected mice and the isolation of the agent from wild rodents and mosquitoes, suggested that AN 17289 might be a strain of Encephalomyocarditis (EMC) virus. Testing with hypertimmune guinea pig serum for the Mengo strain of EMC virus (prepared by dr. Casals in 1956 and supplied by dr. Spence of TRVL) confirmed that AN 17289 is closely related or identical to EMC virus.

The EMC virus was isolated a total of 41 times from 33 infections (Table 1) between January 4 and April 20, 1960. When, in addition to the infections at IAN and Utinga, this agent also appeared in material from horse and bird collected in Bragança, and from mosquitoes at the Brasilia road study area suspicion was aroused that the mouse colony stock might be incriminated. Evidence of the validity of the isolations was sought in a number of ways:

a) the most convincing proof can be found in the testing of sera from the host animal. Pre and postsera were available from two of the four wild rodents from which EMC virus was isolated. The log of the neutralizing index was 2.7 in one case and 3.0 in the other;

b) reisolation of the virus from original material stored at minus 60ºC was effected from liver of three *Proechimys*, from the viscera of a bird, the brain of a horse and from three mosquito suspensions. Not all specimens are available for retest;

c) comparison of the percentages of mouse groups yielding EMC virus after inoculation with original material from

various sources, shows that the rate of isolation was three to five times greater with material from horse, wild rodent, and sentinel mouse than with material from mosquito, opossum, and bird. Results were negative for mouse groups inoculated with material from man or animals other than those mentioned above, and for groups inoculated with sterile broth.

It may be significant that the sources from which the highest percentage of positive groups was obtained (wild rodents and horses) are also the sources in which antibodies have been demonstrated by neutralization testing of survey sera. Neutralization tests with EMC virus and sera from 25 horses showed antibodies in eight samples. Among 34 sera from *Proechimys*, three were positive, and one of ten sera from cows was positive. A total of 41 sera from Jacana, opossum and *Nectomys* was negative.

Complement-fixation testing with EMC antigen and 15 sera from adult colony mice was negative. In another series of 28 adult mouse sera tested by CF, three showed complement fixation at 1:2 buh were negative at 1:4. These were sera from 28 of 72 mother mice who had raised the litters inoculated i. c. with sterile broth in the hope of provoking a latent infection, if present, to become manifest. Two mothers with 12 infants were kept in the same box because of a shortage of boxes at the time. The mice were observed twice daily in the same routine as other inoculated groups and any suspicious or dead infants were passaged. Only three passages were made, none of which gave a virus isolation. A few of the infants disappeared, due to cannibalism, but at no greater rate than was occurring in other groups of 12 infants with two mothers.

This accumulated evidence seems to corroborate the belief that the EMC isolations were from the various indicated sources and not from mouse colony. That the virus was not in the mouse colony is further borne out by the fact that no EMC isolation has been obtained since the apparent cessation of the epizootic in the study areas.

SUMÁRIO

Uma epizootia de vírus Encephalomyocarditis foi estudada no Laboratório de Vírus de Belém. Quarenta e um isolamentos foram obtidos de 33 infecções entre 4 de janeiro e 20 de abril de 1960 de roedores silvestres, marmosa, cavalos, pássaros, camundongos sentinela e mosquitos. Anticorpos foram demonstrados no sangue de roedores silvestres, cavalos e vacas. Êste vírus não foi isolado na área de Belém, antes nem depois dêste período.

SUMMARY

An epizootic of Encephalomyocarditis virus was studied in the Belém Virus Laboratory. Forty-one isolations representing 33 infections were obtained between January 4 and April 20, 1960, from wild rodents, opossum, horses, birds, sentinel mice and mosquitoes. Antibodies were demonstrated in the blood of wild rodent, horse, and cow. This virus was not isolated in the Belém area before nor after this period.

Table 1 – Encephalomyocarditis infections in the Belém area. January to April 1960, by source and location of isolates

Source	Location			
	Bragança	IAN	Km-93	Total
Sentinel mouse group	2	11		13
Wild animals				5
Proechimys guyannensis (3)*		4		
Marmosa sp.		1		
Horse (1)*				2
Bird				4
Jacana jacana (1)*	1			
Monasa nigrifrons	1			
Guira guira	1			
Urubutinga	1			
Mosquitoes				9
Aedes aborealis (1)*			1	
Aedes fulvithorax		1		
Mansonia arribazagae (1)*		2		
Mansonia venezuelensis (1)*		1		
Culex sp.	1	1		
Aede taeniorhychus	1			
Aedes serratus	1			
TOTAL	11	21	1	33

* Reisolation from material preserved at – 60ºC from number host indicated in parenthesis.