PRNC 35

PUERTO RICO NUCLEAR CENTER

TERRESTRIAL ECOLOGY PROGRAM, PART II

RADIATION INDUCED VARIABILITY IN INDIGENOUS ARTHROPOD-BORNE ANIMAL VIRUSES OF PUERTO RICO

PROGRESS SUMMARY REPORT NO. 1

April, 1964

OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT NO. AT (40-1)-1833 FOR U. S. ATOMIC ENERGY COMMISSION

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Progress Summary Report #1
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Support for this program was received in April 1963 but the initial few months were spent in recruiting personnel and training them. Now we have established a mouse colony, holding space and a virus laboratory in the temporary quarters and the program has been activated. It is anticipated that the permanent small animal facility and laboratory will be ready for occupation by fiscal year 1966. Field operations at the study site to date have been oriented towards mosquitoes and rodents. Permanent trap lines have been laid and are set weekly to trap rodents which are marked and released. At selected intervals the rodents are bled for antibody studies and also for attempted virus isolation. Mosquitoes are caught in light traps and also when alighting on human bait. After identification, the mosquitoes are triturated in bovine plasma albumin and the resulting suspension inoculated into mice to attempt virus isolation. To date no virus isolation have been made from material collected at the El Verde Field Station. Between August 13 and December 31, 153 rodents were trapped 618 times - of these, 41 were caught once, 27 twice, 15 three times, 16 four times, 12 five times, 9 six times, 9 seven times, 4 eight times, 10 nine times, 1 ten times, 1 eleven times, 2 twelve times, 2 thirteen times, 1 fourteen times and 2 fifteen times. Each time

a rodent is caught it is weighed and its general condition is noted. It is interesting to note that most animals have a fairly restricted home range but occasionally wander away though inevitably return to the home range.

The female mosquitoes caught on the mountain have been processed as 29 pools. The number of each species inoculated are listed below.

Aedes aegypti	44
Aedes species indeterminate	17
Culex quinquifasciatus	1
Culex nigripalpis	775
Culex species indeterminate	581
Anopheles species	16
Mansonia species	24
Uranotaenia species	1

In August, 1963 a "Dengue-like" illness occurred in an epidemic scale in the town of Manati, Puerto Rico. Manati is situated 3.5 miles inland at the mid point of the northern coast 26 miles west of San Juan. It is probable that cases of the disease occurred elsewhere on the island before the epidemic at Manati; we have, for instance, records of similar illness during September in the staff of the Puerto Rico Nuclear Center

at Mayaguez on the western coast. Shortly after Manati was declared an epidemic area cases occurred in the San Juan area and notably in Bayamon. From here it "moved" to the eastern end of the island and then down the coast with what would appear to be the last cases in the neighborhood of Ponce which is situated to the west of center of the southern coast.

The Puerto Rico Nuclear Center became involved in the measures taken to study the disease when the Arbovirus unit of the Medical Sciences Division was invited to join Dr. Costa Mandry's team from the Department of Health and a team from the Communicable Disease Center, Atlanta, Georgia. On August 23 it was arranged that specimens could be collected from acutely ill patients in Manati and 24 patients were bled by the team of the Puerto Rico Nuclear Center to which Dr. Agustin Cajigas of the Department of Health had been assigned for duty during the investigation.

It may be noted here that this outbreak of illness has resulted in the establishment of an extremely valuable link between the laboratories of the Department of Health and the Puerto Rico Nuclear Center which now work together and cooperate very closely and Dr. Cajigas has been appointed to an Ad Honorem position as Associate

Scientist in the Division. Our connection with the C.D.C. group is of long standing and we are indebted to them not only for assistance currently being received but also for help in establishing the unit here.

On August 29, Dr. Telford Work, Chief, Virology Section at C.D.C. visited Manati with Dr. Cajigas and they managed to find and obtained specimens from 6 individuals previously bled and 5 who claimed to have been afflicted in the past 3-4 weeks. On receipt of these specimens, at about 9:00 P.M., they were immediately processed and set up in an haemagglutination-inhibition test against Dengue type I antigen provided by C.D.C., Atlanta. The erythrocytes were added to the test at 5:00 A.M. and the test read at 6:00 A.M. on August 30. The results are set out below, the results identified by letters pertain to the sera from the individuals with a "history" of disease.

Specimen	Acute serum titer	Convalescent serum titer
#6 F 48	< 1:20	1:80
#10 M 32	< 1:20	1:2560
#12 M 21	< 1:20	1:80
#15 M 22	< 1:20	1:160
#19 M 18	< 1:20	1:160
#22 F 12	@ 1:20	> 1:10240
A F		1:640
B F 65		> 1:10240
C F		1:80
D F		< 1:20
E M 70		< 1:20

From these results it was clear that the disease was associated with an agent which is related to Dengue I type virus. (Until such time as an agent is adapted to regularly kill a laboratory animal, it is not possible to establish its exact identity). The agent which caused the illness in Puerto Rico, in all probability is the same as that which caused the epidemic in Jamaica a few weeks prior to the one here, has proved unusually hard to adapt to either laboratory mice or tissue culture. Several different groups have been working with it but none

have had any success in fully adapting an agent from many samples of material, though as described in the last paragraph our unit would appear to have had the greatest success.

The contribution of the Puerto Rico Nuclear Center to the overall effort lay largely in the initial preparation and screening of specimens collected in the field, in all 1,237 human blood specimens were centrifuged, the serum separated and ampouled, one ml of each serum was also treated for testing by the haemagglutination-inhibition test. Aliquants of all specimens were sent to C.D.C., Atlanta both for attempted virus isolation and serological study.

Of the 1,237 sera 88 were taken in the first 6 hours of illness and in this laboratory were inoculated into infant mice and 143 taken in the first 12 hours into tissue culture to attempt virus isolation. Mosquitoes numbering 17,943 were processed in 180 pools - the distribution by species is shown below.

A. aegypti	6,849
C. quinquifasciatus	10,983
C. nigripalpis	26
Culex species	85
	17,943

These mosquitoes were caught by a team consisting of our own field staff augmented by some catchers from the Department of Health, all under the supervision of Mr. Leslie Beadle of the C.D.C. staff, who was also responsible for their identification. The human sera came from a variety of sources, mainly those collected by a team from the Epidemiology Branch at C.D.C. and from an obstetrician, Dr. Arandes, who has provided 129 paired sera from pregnant women who had the disease in order to study any possible effect these may have had on the foetus.

From 22 acute blood specimens and from 30 pools of mosquitoes, agents have been obtained which cause transient illness in infant mice and 38 a cytopathogenic effect in African Green monkey kidney tissue cultures. The presence of virus may be shown in both infected mouse brain and tissue culture cells by means of the indirect fluorescent antibody technique. In the hopes of increasing their pathogenicity, two strains of virus are in continuous passage in infant mice and two more are in serial passage in infant mice which have been irradiated with an air dose of 300 rep of X Ray (from a 350 KVP machine) prior to inoculation in order to decrease their natural resistance to the agent. A

well controlled experiment has given extremely encouraging results in that the irradiated infant mice inoculated with serum from an acutely ill patient sicken and die and their brains give a strongly positive reaction by the fluorescent antibody technique. After 2 further passages it is hoped to achieve production of antigens for use in serological tests which are of sufficiently high titer to be of value in achieving from identification of the agent by reciprocal tests with the 6 known dengue viruses. A haemagglutin has been made from 2 strains of virus, but its potency is so low that it is of little value for practical purposes.