The use of a micro hemagglutination-inhibition test to follow antibody response after arthropod-borne virus infection in a community of forest animals*

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The techniques detailed for hemagglutination-inhibition arthropod-borne viruses by Clarke and Casals[1] in 1958 have been adapted to the microtiter method of Takatsy as modified by Sever[2]. The resulting technique has sensitivity and accuracy comparable to methods using larger volumes and saves considerable time and reagents.

This micro method was found ideally suited to the study of antibody of forest animals which were captured, marked, released, and recaptured in the Utinga forest near Belém, Brazil. Details of the trapping methods and virus isolation results are being presented by dr. Calista Causey in another section of this program.

Antigens for the study were made from 22 viruses indigenous to Brazil by the sucrose-acetone technique for mouse brain, or by two acetone extractions for infected mouse serum. A working antigen suspension containing approximately 16 units was made in 0.4% bovine albumin borate buffered diluent at pH9. This suspension was stored frozen at -60°C, thawed to withdraw the amount needed for the day's testing, and refrozen. A slight drop in titer was noted with some antigens after repeated freezing and thawing, but no loss of sensitivity

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occurred as judged by the titer of a homologous serum included in each test. Goose cells, 1:200 were used for hemagglutination.

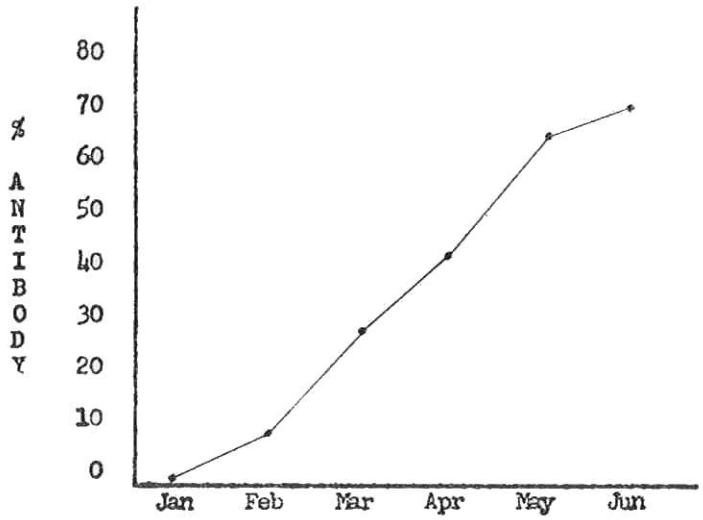

Fig. 1 – The incidence of group B (Bussuquara) HI antibody in the spiney rat of Utinga forest, January through June, 1963.

N° animals	26	24	24	26	39	43
Antibody conversion	1	5	4	7	2	–
Bussuquara isolation	0	0	1	0	0	0

Non-specific inhibitors of sera were acetone extracted by the reference method of Clarke and Casals[1], using 0.05ml of serum measured from a calibrated dropper. This was enough for testing with 22 antigens. Attempts to dilute the sera to 1:4 instead of the recommended 1:10 in saline before extraction and thus use less acetone, were abandoned because, although satisfactory for removal of non-specific inhibitors of group A viruses, they were not satisfactory with group B viruses. To remove goose cell agglutinins, rehydrated sera in the 1:10 dilution were adsorbed with a 1:6 suspension of goose cells in bovine albumin diluent using 0.6ml of cell suspension per 0.5ml of 1:10 serum.

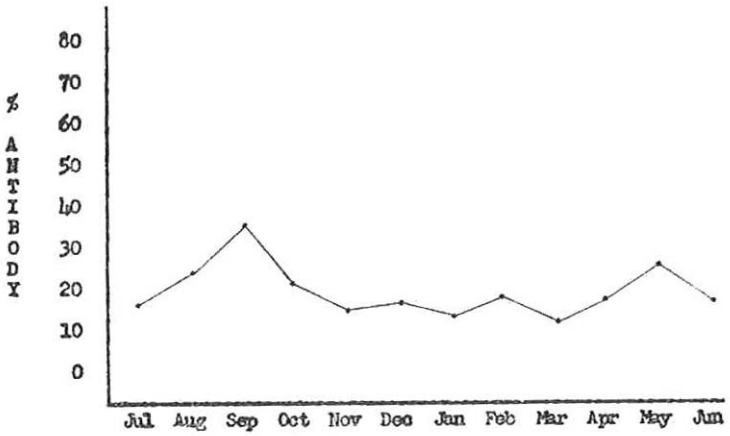

Fig. 2 – The Incidence of group A (Mucambo) HI antibody in the spiney rat of Utinga forest, July, 1962 through June, 1963.

N° animals	36	35	37	47	45	42	26	24	24	26	39	43
Antibody conversion	0	0	1	0	0	1	0	2	0	1	2	3
Mucambo isolation	0	1	0	0	0	0	0	0	0	0	0	0

The resulting 1:20 serum dilution was used for screening with four units of antigen in microtiter plates described by Sever[2]. End-points were determined on positive sera using microtiter loops[2]. Twenty-five thousandths ml each of serum and antigen and 0.05ml of cell suspension were added by calibrated dropper. Cells were mixed by turning the plate almost vertically and tapping on the table top.

Using this technique, one person completed about 3,000 determinations per week. The type and usefulness of the epidemiological information obtained on minute quantities of serum from rodents and marsupials is illustrated in the 1st figure. Bussuquara virus was not active in the Utinga forest between July, 1962 and January, 1963 as judged by hemagglutinating-inhibiting antibody of the forest animals. No group B antibody was detected in January bleedings. In February, group B antibody, characteristic of that following Bussuquara infection, was noted in 8% of the spiney rat, *Proechimys guyannensis oris*. The level

rose progressively until in June, 70% of this species had group B antibody. None of seven other species sampled had significant group B antibody. During this six months, 20 spiney rats showed antibody conversion and Bussuquara virus was isolated from the blood of one in March. This rapid climb in antibody level is interpreted as resulting from an epizootic of Bussuquara virus in spiney rats of Utinga forest.

The second figure shows the hemagglutinating-inhibiting antibody incidence in the same population, of Mucambo virus, which is closely related to Venezuelan Equine Encephalitis virus. In contrast to Bussuquara virus, Mucambo is thought to have been enzootic during the period of the study. The incidence of detectable antibody ranged between 13 and 35% with monthly fluctuations. Mucambo virus was isolated from the blood of a spiney rat in August and antibody conversions were recorded throughout the year. In addition, six of the seven other species sampled had Mucambo hemagglutinating-inhibiting antibody.

SUMMARY

The use of a microtechnique is described for hemagglutination-inhibition testing for arthropod-borne viruses. Testing for 22 viruses was carried out with 0.05ml of serum. The results of testing with Bussuquara and Mucambo viruses for antibody responses in serial bleedings of small forest animals captured and recaptured in Pará, Brazil, are discussed.

REFERENCES

1. CLARKE, D. H. and CASALS, J., 1958 – Techniques for hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. & Hyg., *7*: 561-573.

2. SEVER, J. L., 1962 – Application of a Microtechnique to viral serologicaI investigations. J. Immunol. *88*: 320-329.