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BRIEF NOTE

DUGBE VIRUS REPLICATION IN NYMPH AND ADULT *AMBLYOMMA VARIEGATUM*

J.P. Gonzalez (1), **J.P. Cornet** (2) and **J.L. Camicas** (2)

(1) *Institut Pasteur, BP 220, Dakar, and*
(2) *Laboratoire ORSTOM de Zoologie médicale, Institut Pasteur, Dakar*

INTRODUCTION

Dugbe (DUG) virus is a member of the Nairobi sheep disease serogroup (genus *Nairovirus*, family *Bunyaviridae*). Crimean Congo haemorrhagic fever (CCHF) virus belongs to the same group and presents some antigenic similarities with DUG virus (Casals and Tignor, 1980). Nevertheless, DUG virus infection in humans is rare and weakly pathogenic, while CCHF virus is responsible for severe human epidemics (Digoutte *et al.*, 1980; Hoogstraal, 1979). Because of the few restrictions in handling DUG virus in the laboratory, it has been proposed and used as a candidate for developing CCHF diagnostic techniques and as a model for understanding the ecology and molecular biology of nairoviruses (Nuttal P.A., pers. comm.; Cornet *et al.*, 1987; Huard *et al.*, 1978).

Because of our involvement in a long-term study in a CCHF epidemiological research programme, DUG virus was studied as a model principally to improve new laboratory techniques. The present work was aimed at determining the most efficient tick stage for DUG virus replication with experimentally infected tick colony specimens.

MATERIALS AND METHODS

Virus strain.

A Nigerian strain (IbAR 2484) of DUG virus, isolated from *Amblyomma variegatum* in Ibadan, was used (Berge, 1975). The 12th passage through mouse brain was titrated on suckling mice (titre = 6.4 LD_{50}/0.02 ml).

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Tick colony.

A. variegatum was chosen because it is the common vector of DUG virus (Camicas, 1980). The colony had been initiated, from eggs of a blood-fed female tick taken from cattle at the Bangui abattoirs in 1986 (Central African Republic). To exclude any viral contamination, one half of the resulting nymphs were inoculated into 24-h old suckling mice. New generations of nymphs were tested periodically in a similar manner. Nymphs and adults inoculated in the present experiment were used after F2 progeny.

Intracoelomic inoculation.

A basic technique, previously described was used with the following changes (Lei and Kemp, 1970; Stelmaszyk, 1975): (a) a 0.5-µl virus suspension was inoculated into the ventral surface of nymphs and (b) 7.0-µl of the same suspension was inoculated by the intra-anal route into adult ticks. The volumes used for inocula avoided any spill during inoculation.

Two concentrations of virus supension were tested: undiluted and diluted to 10^{-4}. A total of 300 nymphs and adults were inoculated with undiluted virus and the same number was inoculated with diluted suspension. From day 0 to day 14 post-inoculation (p.i.), 10 nymphs and 5 adult ticks were removed from each pool and ground in Hanks medium for virus titration by intracerebral (i.c.) inoculation of 1-day old mice.

Virus titration.

Ground mice brains and ground ticks were titrated by i.c. inoculation into 1-day old mice and the titre expressed by a decimal logarithm of suckling mice lethal dose 50 %.

RESULTS AND DISCUSSION

The virus eclipse phase, for both nymphs and adults, was shorter for the undiluted inoculum (24 h) than for the diluted one (5 to 9 days) (table I). Between days 3 to 10 p.i., the virus titre reaches a plateau.

Virus replication appears to be more efficient in the adult tick, and titre gain is obtained only in experiments using diluted inocula showing an increase in virus titre of 2.2 LD_{50}/0.02 ml in adult and 1.6 LD_{50}/0.02 ml in nymphs.

On day 9 p.i., there was no significant difference in titre between nymphs and adults regarding inoculum titre ($X^2 = 0.224$, $p = 0.636$). Nevertheless, because of a lack of gain in virus replication in experiments using undiluted inocula, it seems that high virus titres induce interference phenomenon impairing replication efficiency in ticks.

CCHF = Crimean Congo haemorrhagie fever.
DUG = Dugbe (virus).
i.c. = intracerebral(ly).
p.i. = post-inoculation.

TABLE I. — **DUG virus replication in nymph and adult ticks. (*A. variegatum*) experimentally infected.**

	Nymph		Adult	
Days p.i.	Undiluted (*)	10^{-4}	Undiluted	10^{-4}
0 (2 h)	1.6 (**)	0.0	1.3	0.0
0 (6 h)	1.6	0.0	1.4	0.4
1	0.0	0.0	0.0	0.0
2	3.2	0.0	2.4	0.0
3	4.4	0.0	2.4	0.0
4	4.4	0.0	2.8	–
5	–	–	4.0	0.7
6	4.0	0.0	5.0	5.1
7	–	–	4.4	5.6
9	3.4	3.0	5.2	5.6
10	–	3.0	6.0	–
12	–	–	5.4	–
14	–	–	5.2	–

(*) Inoculum titres: undiluted = 6.4 LD_{50}/0.02 ml; 10^{-4} = 2.4 LD_{50}/0.02 ml.
(**) Virus titre LD_{50}/0.02 ml.
– = Not tested.

We can conclusively assume that only a limited number of permissive cells are infected, resulting in a plateau of the virus multiplication kinetics in the tick. This way, the virus can replicate more intensively in adult with 20- to 22-fold the size of nymphal ticks (J.P.C., unpublished data). Our hypothesis does not implicate any role played by physiological differences between the tick stages (Okorie and Fabiyi, 1979).

Okorie and Fabiyi (1979) used *Hyalomma marginatum rufipes* ticks in a similar experiment. They showed that nymphs replicate DUG virus more efficiently than adults with a 65 % increase in viral titre. Moreover, the viral titre of *H. m. rufipes* nymphs was 43 % higher than that obtained from *A. variegatum* adult ticks in our experiment. Despite their claim to have inoculated 20 µl into each nymph and adult tick (an unrealistically large quantity), the viral concentration was similar to the diluted inoculum which we used in our experiments. Inoculation route could not have affected virus replication because in both cases virus was injected directly into the coeloma. In conclusion, the explanation for such discrepancies between our observations and the previous ones, if real, should be found only in tick strain genetics and/or virus variations.

Studies are in progress, using different strains of CCHF virus and tick species, to clarify eventual variability in the eclipse phase and the ability of nairoviruses to replicate in ticks.

KEY-WORDS: Arbovirus, Dugbe virus, *Amblyomma variegatum;* Replication.

RÉSUMÉ

RÉPLICATION EXPÉRIMENTALE DU VIRUS DUGBE CHEZ LA NYMPHE ET L'ADULTE DE LA TIQUE *AMBLYOMMA VARIEGATUM*

Des spécimens d'adultes et de nymphes de tiques (*Amblyomma variegatum*) colonisées au laboratoire ont été infectés expérimentalement avec deux préparations de virus Dugbe. La phase d'éclipse du virus chez la tique augmente avec la dilution de la suspension virale. La réplication du virus est supérieure chez les adultes par rapport à celle observée chez les nymphes. Le titre viral après le 9e jour post-inoculation est sensiblement le même quel que soit le titre de l'inoculum initial; toutefois, un inoculum de titre élevé entraînerait un phénomène d'interférence, diminuant d'autant le pouvoir amplificateur de la tique sur le virus.

MOTS-CLÉS: Arbovirus, Virus Dugbe, *Amblyomma variegatum;* Réplication.

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