

(6559)

année: 1996

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Running title: Ecology of amazonian arboviruses

Key words: Arboviruses - Ecology - Hosts - Amazonia - South America

Ecological aspects of arboviroses in Brazilian Amazonia, South América¹.

Abstract: Ecological data on the known hosts of 186 arbovirus species which have been shown to occur in Brazilian Amazonia are examined. In order to make an ecological classification of viruses, according to the ecology of their hosts, a multi-dimensional niche concept has been retained. Both vertebrate and invertebrate hosts have been separated by their soil vs. canopy preferences and diurnal vs. nocturnal behavior. It has been verified that the number of hosts involved in transmission cycles (ecological diversification) seem to be independant from the systematic diversification of the viruses. Another interesting fact is that each serological group has but one virus in one ecological group, probably meaning that serological constraints are at work during its ecological diversification. The main result of our studies concerns the dynamic equilibrium which characterizes communities. Perturbations of this equilibrium, and the fairly quick recover of a new one, have been shown to occur during the construction of big dams in Amazonia. The needs for future researchs are, among others: the hosts of 2/3 of the viruses, the variations of the virus community along time and the genetics of emergent types.

Introduction

The amazonian region is probably the world's richest reservoir of arboviruses. The 186 different types of arboviruses so far detected in the brazilian part of the region (1-6) and Travassos da Rosa (unpubl. data) account for above one third of the world's arboviruses (537) (7). Of these, 136 (83.4 %) are endemics in this part of the neotropical zoogeographical region.

¹Many aspects of this work have been presented at the "Sixth Symposium on Arbovirus Research in Australia, December 7-11, 1992, CSIRO/QIMR, Brisbane, Queensland, Australia"



The virological and serological data, obtained between 1954 and 1992 at the Evandro Chagas Institute / National Health Foundation (Belém)², have been analysed from two complementary points of view: (i) the definition of the ecological niche of each arbovirus and (ii) the ecological factors which have possibly constrained their evolution.

The ecological niche concept has been used recently by Calisher (8) in relation with the definition of the virus species: "A virus species is a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche (emphasis by us)". A definition of the ecological niche which best suits to the arboviruses and has been used once by Dégallier *et al.* (9) is: "A niche is a multi-dimensional hypervolume of resource axes" (Colinvaux (10), p. 31). In the case of arboviruses, each host or alternatively, each component of the hosts' environment may represent one of these resource axes or variables, allowing for the quantification of the niche. Thus, various data analysis methods revealed themselves particularly well adapted (i) to the numerical definition of the niche of each virus species, (ii) to the study of the ecological grouping of the viruses, and (iii) of the man-made modifications of the natural environment which may cause the emergence or re-emergence of arboviruses (11,12).

Another question which is not yet resolved but is related to the above two points is: what are the ecological factors which are preventing arboviruses from multiplying randomly in all available hosts? There are probably constraints of various origins and located at various levels, from inside the cell to the ecosystem: genetical (or physiological) (13), eco- ethological and historical (or biogeographical) (14).

The crude data was represented by the

isolated strains and the results of haemagglutination inhibition (HI) tests (the laboratory techniques are described in detail in Shope & Sather (15)). In order to avoid the introduction of false positives in the data, even at cost of some false negatives, the HI tests have been considered positive for a particular arbovirus if it showed a monotypic reaction or a titer at least four-fold above any of other tested antigen in the same serological (cross-reacting) group (A.P.A. Travassos da Rosa, unpublished results).

Table 1: Numbers of genera, serological groups and species for each family of arboviruses present in the amazonian region of Brazil; their order of enumeration follows decreasing number of species.

| Family | genera | groups | species |
|-----------------------|--------|--------|---------|
| Bunyaviridae | 2 | 11 | 70 |
| <i>Bunyavirus</i> (a) | - | 10 | 45 |
| <i>Phlebovirus</i> | - | 1 (b) | 25 |
| Reoviridae | 1 | 2 (c) | 63 |
| Rhabdoviridae | 1 | 5 (d) | 15 |
| Togaviridae | 1 | 1 | 8 |
| Flaviviridae | 1 (e) | 1 | 8 |
| Coronaviridae | 1 | 1 | 1 |
| Poxviridae | - | - | 1 |
| Unclassified | - | - | 15 |
| (Arenaviridae) (f) | 1 | 1 | 3) |
| (Herpesviridae) (f) | - | - | 1) |
| (Paramyxoviridae) (f) | - | - | 1) |

(a) with 1 *Bunyavirus*-like virus included

(b) with 4 ungrouped viruses included

(c) with 3 ungrouped viruses included

(d) with 3 ungrouped viruses included

(e) with 1 ungrouped virus included

(f) probably not arboviruses

An intuitive knowledge about the intensity of the adaptive radiation of arboviruses in Amazonia is provided by both the host spectrum of each virus (table 1) and number of viruses

²The following figures will give an idea of the sample which have furnished the data which formed the base of our present knowledge (Nr. of specimens or pools): haematophagous Diptera, more than 515000 pools; marsupials, 6427; bats, 9276; wild monkeys, 2428; rodents, 18741; edentates, 861; carnivorous, 361; ungulates, 3374; birds, 12423; reptiles, 6052; amphibians, 1509.

Table 2: Minimum numbers of different species of vertebrate and arthropod hosts for each family and/or genus of arboviruses present in the amazonian region of Brazil; their order of enumeration follows decreasing total number of species.

| Virus Family/Genus | Min. Nr. of different species | | Total |
|--------------------|-------------------------------|------------|-------|
| | Vertebrates | Arthropods | |
| Flaviviridae | 37 | 20 | 57 |
| Togaviridae | 39 | 17 | 56 |
| Bunyaviridae | | | |
| <i>Bunyavirus</i> | 15 | 39 | 54 |
| <i>Phlebovirus</i> | 9 | 3 | 12 |
| Reoviridae | 2 | 12 | 14 |
| Rhabdoviridae | 3 | 6 | 9 |
| Coronaviridae | 1 | - | 1 |
| Poxviridae | 1 | - | 1 |
| Unclassified | - | 2 | 2 |

found in each host (table 2). However, the viruses' families with greater number of species are not necessarily those which were found in the greater variety of hosts. For example, the Flaviviridae and Togaviridae with each only 8 viral species in the Brazilian Amazon region, have been found associated with at least 57 and 56 different hosts, respectively, numbers only a little above the 54 hosts known for the *Bunyavirus* which include 45 species. On the other hand, the Reoviridae, accounting for 63 different viruses, are known from only 14 different species of hosts. Thus, the ecological diversification, in terms of number of hosts involved in transmission cycles, seems to be independent from the systematic diversification

of the viruses (= number of species).

Table 2 shows the number of different species of viruses found in each ecological type and/or systematic group of hosts.

Some types of hosts seem to be more favorable to the speciation of arboviruses than others. Among the arthropods, sandflies are almost the sole hosts for the majority of Reoviridae known from our region. As these viruses do not form agglutinins in vertebrates, it is not yet possible to know if they are diversified in this respect. Due to their minuteness and the lack of identification key for fresh females, the Phlebotomine sandflies were not identified and thus, may contain many species with various habits. On average, nocturnal mosquitoes harbour more different viruses than diurnal ones do. This difference results mainly from the number of Bunyaviridae transmitted by these two types of mosquitoes. Among the vertebrates, the same may be said i. e. that the nocturnal ones harbour a larger variety of viruses, due especially to the predominance of the Bunyaviruses. The Flaviviridae seem to be as "diurnal" than "nocturnal" but the Togaviridae may be more "diurnal" if we consider their vertebrate hosts (table 3).

THE MULTIDIMENSIONAL ECOLOGICAL NICHE

The data, under the form of a contingency table, may be explored either by ordination (factorial analysis of correspondances) or classification (ascendent hierarchical

3: The hosts of sylvatic arboviruses in Brazilian Amazonia, grouped according to their ecological characteristics. For each type of host is indicated the number of virus species associated with.

| Arbovirus Families | Mosquitoes | | Sandflies | Midges | Ticks | Vertebrates | | Nocturnal | |
|--------------------|------------|---------|-----------|--------|-------|-------------|------|-----------|------|
| | Nocturnal | Diurnal | | | | terr. | can. | terr. | can. |
| Flaviviridae | 4 | 4 | 0 | 0 | 0 | 6 | 6 | 6 | 6 |
| Togaviridae | 6 | 6 | 0 | 0 | 1 | 6 | 7 | 5 | 5 |
| Bunyaviridae | 37 | 19 | 2 | 2 | 2 | 13 | 17 | 21 | 17 |
| Reoviridae | 8 | 5 | 51 | 0 | 0 | 0 | 1 | 2 | 0 |
| Rhabdoviridae | 3 | 3 | 3 | 0 | 0 | 4 | 2 | 1 | 1 |
| Coronaviridae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Poxviridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Unclassified | 4 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 3 |
| Total | 63 | 40 | 69 | 4 | 3 | 36 | 40 | 45 | 38 |

classification) methods (AnaMul and ADDAD packages, respectively) (16,17). In order to study more specifically the ecological relationships existing between the arboviruses, the hosts have been grouped according to (i) their vertebrate vs. arthropod nature and (ii) their known ecological preferences, e.g. their terrestrial/ arboricolous and diurnal/ nocturnal habits. A more detailed study of the bird-associated arboviruses, based on data about habitat and level preferences of the hosts has been done separately by Dégallier *et al.* (9).

As already shown (18), the arboviruses may thus be grouped ecologically according to the predominance of different types of hosts:

- Nocturnal terrestrial vertebrates/ Nocturnal mosquitoes: BEN, ICO, BSB, MOJU, ACA, BSQ, CAR, CAR-like, CATU, MUC, NEP, CAP, GMA, BVS, ITQ, MUR, ORI, BIM, GJA, GAM, AURA, Trombetas;

- Diurnal terrestrial vertebrates/ Diurnal mosquitoes: UNA, MCA, ILH, TNT, KRI, MAG (+ KWA-like, ANU ?);

- Diurnal canopy vertebrates/ Diurnal mosquitoes: YF, GRO, MAY, SLE, TCM, TUR, WEE, ORO, TCM, JUR;

The viruses EEE and PAC-like seem to localize at intermediate positions, between diurnal and nocturnal and between canopy and ground-dwelling hosts.

Although the above categorization may be useful, it remains unrealistic because there is rather a continuum (or gradient) from "diurnal" to "nocturnal" viruses and from "arboricolous" to "terrestrial" ones. Some viruses as GAM, WEE, TUR, ACA Trombetas and GRO are intermediate, being isolated also from "Nocturnal mosquitoes". It is interesting to note the opposition between the predominantly "diurnal" and "terrestrial" PIX, TNT and MAG viruses, and the almost strictly "canopy-liking" viruses MAY, ILH, YF and UNA. The ecology

of some viruses like ORO, ANU, Tapara, KRI and ICO needs more informations to be gathered, especially about their vectors.

With few exceptions, each serological group has but one virus in one ecological group; however, we need finer definitions of the niches of 21 viruses pertaining to A, B, BUN, C, CAP, CGL and GMA serological groups.

In a special study, Dégallier *et al.* (9) considered a subset of the data which included 30 different bird-associated arboviruses. In this case, more precise ecological variables have been used to classify the viruses, namely five types of vegetation, two of which (igapo or inundated forest and "terra firme" forest) has been subdivided in two and five strata, respectively. A gradient has been observed between the birds' species which are preferring secondary vegetation or forest (= "capoeira") and, those which are found mainly in primary forest.

The viruses CPC, MAY, ILH and TCM have an important secondary forest component (25 %). The birds which are the hosts of UTI, KWA and GAM viruses are species living exclusively in the "terra-firme" forest.

EEE virus has been "found" in all but one rare type of vegetation or strata (forest on sandy ground) and is considered as ecologically versatile. This may be linked to a great potential of this virus to colonize new niches, including in urban environment³.

The viruses which may be considered the more prone to infect human people in rural places are GMA, MUC, MUR and APEU because they have been found in birds which are living in the secondary (or degraded) environments mixed with cultivated areas as is often the case in Amazonia. They are actually fairly prevalent in human sera.

DISCUSSION AND CONCLUSIONS

Previous works have already described the

³Some strains have been isolated from mosquitoes collected in the suburbs of Fortaleza city, Ceará, Brazil

probable sylvatic cycles of many amazonian arboviruses (1,19,20). However, the grouping of ecologically similar viruses was made mainly after the number and nature of the hosts, i. e. the relative "complexity" of the cycles. We have reexamined the same data with quantitative methods. In a quantitative ecological study of the viruses pertaining to the group C of *Bunyavirus*, Woodall (21) has shown a marked niche separation between canopy- and ground level-transmitted viruses and, when two viruses appeared to share the same niche, that the vectors were distinct species. Thus, for establishing themselves in a locally stable equilibrium, related viruses cannot share the same arthropod and/or vertebrate hosts. It may even be said that the less they are serologically related, the more they can share the same ecological niche.

The complexity of the amazonian environment, exemplified by an extreme diversity of vertebrate and arthropod species, and consequently of niches, have favoured the diversification and therefore sympatry of many arboviruses of the same group. As our studies have shown, the presence of two or more different viruses of the same serological group in what has been characterized as one niche may be due to the coarseness of the ecological variables which have been considered. As many ecologists have shown, temporal and spatial variables may also be included in the multidimensional definition of the niches. The isolations of strains and/or serological conversions in sentinel animals should be interpreted in the future for the "temporal" characterization of the niches.

Excepted for some viruses which seem to be ecologically very distinct of all others (for ex. PAC-like (22)), there is no clear-cut separation between one group and the next along the "ecological transects" defined either by the preferred vegetation types and strata, or by the habits of the hosts. This may mean that, with similar historical (= biogeographical + genetical) constraints, the arboviruses' population in a defined community forms a dynamic equilibrium. Subsets of this population may

share the same niche, at least at the two levels studied here, and each niche is separated from the others by ecological constraints acting on the hosts' populations. However, two very different types of perturbations can modify this equilibrium. When some fluctuations are going on in hosts' populations, as occurs seasonally for non-immune hosts, only the arbovirus transmission levels are affected. On the contrary, when the hosts' populations are permanently modified, the equilibrium of arboviruses' populations need to shift to a new state. Eventually, new niches may appear which would be filled after a short time. These may be colonized by new genotypes, obtained by recombination, reassortment or introduction from adjacent communities (8). Rapid adjustments of equilibrium have been noted in the case of the important perturbations induced by the filling of a dam reservoir, where "new" arboviruses appeared in the area, either as exogenous material or as autochthonous speciation (23).

The phylogenetic study of arboviruses, based on the viruses-hosts associations is yet very tentative because of the lack of phylogenetic classifications of either groups (24). This author has looked for some evidence of a host-parasite coevolution in the case of the California serogroup viruses. What we have defined as historical constraints are doubtlessly related with some effects of coevolution but it remains difficult to distinguish these from the constraints arising from viruses competition after horizontal transfers (25). These hypothesis need to be evaluated by molecular biologists.

What happened with the viruses for which some evolutionary hypotheses are available, based on protein sequencing? Levinson *et al.* (26) furnished some interesting hypothesis which will be discussed from an ecological point of view. MAY (with Una, not studied by these authors), EEE/WEE, AURA and MUC (member of VEE complex) seem to have diverged in four different directions. As other authors have shown, WEE virus arised probably as a

recombinant between EEE- and Sindbis - like ancestors (27,28). EEE seems to be a very versatile virus, especially in birds whereas HI antibodies against AURA have been found in rodents (29). The ecological link between the two may be the Melanoconion and Ochlerotatus subgenera of Culex and Aedes mosquitoes, respectively.

The available data are not detailed enough to explain why some viruses of the same serological group coexist and other do not, but it allowed us to define some main ecological groups. Each of these groups further needs to be studied separately as it has been done with the bird-borne arboviruses.

Despite the great number of tested pools between 1954 and 1992, serological and virological data are lacking either for viruses which do not form agglutinins, or about potential hosts which are difficult to collect. In fact, less than one third (28.9 % or 50/173) of the viruses known from sylvatic hosts have been found in both vertebrate and arthropod hosts, 17.7 % (29/173) are known only from vertebrate hosts and 54.3 % (94/173) only from arthropods. Among the latter, 63.8 % (60/94) are known only from phlebotomine sandflies and represent probably a very complex ecological system.

Sampling bias may account for some distortions in the quantitative delineations of the niches. For example, ground dwelling rodents, marsupials and birds are much easier to trap than canopy frequenting hosts and among them

those which cannot be attracted by any type of baited trap. Thus, many species are poorly known, not only for the viruses they may harbour but also for their bioecology. An important ecological "axe" which has been yet neglected is the time or seasonal one. It is quite conceivable that some hosts may harbour different viruses of the same serological group at different times of the year. This may be especially the case with bunyaviruses whose antibodies are not life-long lasting (30). In future studies, the interpretation of serological tests ought to be fine-tuned, according to each virus-host association.

Nevertheless, quantitative multifactorial analysis seemed adequate for the study of the multidimensional niche concept of arboviruses, and it will also reveal itself a useful tool to make predictions about the natural evolution of the arboviruses in response to modifications of the natural environments.

Acknowledgments



We wish to thank all staff members, both from the Evandro Chagas Institute and other Institutions, for their past and present participation in the field and laboratory work which has made this paper possible. The present work has benefited of the unvaluable financial and/or logistic help of Eletronorte (Eletrobras), SUDAM (Polos Agropecuarios da Amazonia), CNPq, ORSTOM and Foundation SESP (now National Health Foundation, Ministry of Health).

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 **C.K. Comércio e Representações Ltda.** 

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