PONTIFICIA
ACADEMIA
SCIENTIARVM

COMMENTARII

Vol. II N. 44

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EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

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Vol. II - N. 44
pag. 1-16

BAT MALARIA: ZOOGEOGRAPHY AND POSSIBLE COURSE OF EVOLUTION

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SVMMARIVM — Auctor problemata de distributione febrium illarum quam « malaria » (malus aër) vocari solent, in chiropteris exponit et discutit. Infectio in mundo antiquo frequens est, in novo vix nota. Pauca tamen exempla polychromophilorum in America nota sunt, quod naves chiroptera infecta aut ex Europa aut Africa recenter importaverunt. Nihilominus evolutionem chiropterorum, nycteribiorum, polychromophilorum tempore tertiano probabiliter coepisse dicunt.

The subject of the evolution of protozoal parasites is not only highly speculative because of the absence of fossil remains, but complicated owing to the co-existence of vertebrate or invertebrate hosts and frequently of both. It is often assumed that host and parasite evolved together; thus, BARUŠ and RYŠAVÝ (1971) point out that the phylogenetic development of nematodes and bats proceeded along parallel lines. This idea may well be incorrect and the parasite may have arrived on the scene at a later date, either from a free living ancestor or adapted with a new cycle from a totally different host. Bat malaria represents a particularly interesting exam-

Paper presented on April 14th, 1972 during the Plenary Session of the Pontifical Academy of Sciences.

ple, because of its curious distribution and the different forms which it has assumed. Moreover in one form, the parasite (*Polychromophilus*), the bat and the vector (nycteribiid fly) spend practically their entire lives together and concurrent evolution of the three components, after a certain epoch, may then have occurred. « Bat malaria » rather than « bat malaria parasites » is thus the more appropriate term to employ in this context.

The parasites comprise four genera of pigmented organisms which live in the red blood cells and tissues of insectivorous and fruit bats and their respective invertebrate hosts. Three of the genera (*Plasmodium, Hepatocystis* and *Nycteria*) are confined to the Old World tropics, the fourth (*Polychromophilus*) is cosmopolitan in insectivorous bats, though until recently this genus was thought to be absent from the New World, where is is apparently rare. Species of *Plasmodium* (the true malaria parasite) have so far only been found in tropical Africa; *Hepatocystis* is widespread in the Old World tropics and both these genera are confined to fruit bats. *Nycteria* has only been identified with certainty in insectivorous bats of tropical Africa, but its distribution is suspected of being wider.

The habits of the invertebrate hosts present a key to the zoogeography. *Plasmodium, Hepatocystis* and *Nycteria* are all transmitted by dipterous insects which bite the host in order to obtain blood and then fly off to complete their life cycle elsewhere; the cycle of the parasite also has to take place in this insect, and a high temperature is necessary for its progress. Only in the tropics is the climate warm enough, and these three genera are thus confined to the tropical regions. On the other hand, *Polychromophilus*, is transmitted by nycteribiids, which spend nearly all their life in the hair of the bats, and conditions here are suitable in most parts of the world for the development of the parasite; thus the infection is found equally in temperate and tropical zones. The zoogeography of *Polychromophilus* is not temperature-dependent; some other factors are concerned which offer a challenge to the investigator, and this paper is largely directed to a solution of the problem. Firstly, however, a brief account is given of the distribution of species of the three tropical genera. This classification was presented by the author (GARNHAM 1953, a & b) to the VI Congresso Internazionale di Microbiologia in Rome in 1953.

Two species of *Plasmodium* occur, and both are found in Africa. *P.roussetti* is a rare parasite of roussettes (fruit bats) living in the caves of Mont Hoyo in the eastern Congo, and is probably transmitted by a special cave-haunting species of *Anopheles*. *P.voltaicum* is found in *Roussettus smithi* occupying cavities in the banks of rivers in Ghana, and is transmitted by *A.smithii*. Other strains of this species probably occur elsewhere on the West Coast (e.g. near Brazzaville and Cameroun).

The genus *Hepatocystis* is much more widespread than *Plasmodium*, and species (again confined to fruit-bats) are found over vast regions of tropical Africa, Asia and Australasia. *Epomophorus* is the common vertebrate host in Africa, and *Pteropus* in Asia and Polynesia. Although the latter bats extend to Mauritius, the *parasite* is absent on this island, though present in the Seychelles. The vector is probably some species of *Culicoides*. The habitat of these fruit bats is chiefly trees, but some live in caves; the arboreal cover long distances in seasonal migrations. *Nycteria* is a parasite of insectivorous bats of tropical Africa and possibly of Asia. It was originally described from *Nycteris* a bat living in coral caves on the East African coast, and has a specialised type of exoerythrocytic schizogony in the liver (GARNHAM and HEISCH, 1953). Anciaux de Faveau described a parasite in *Rhinolophus* in the Congo under the name of *Polychromophilus congolensis*, which the author (1966) removed to the genus *Nycteria*, on account of the characteristic development in the liver. However, ADAM and LANDAU (1972) found further examples of this parasite in the same genus of bats in Brazzaville, with sporogony in nycteribiids and the classification of this group may need amendment.

Polychromophilus

Malaria parasites of bats have interested zoologists ever since the day when GRASSI suggested to his younger colleague, DIONISI, that he should abandon the study of malaria parasites of man and examine instead those of bats. DIONISI responded enthusiastically, realising the great advantages which a parasite of small mammals could offer for malaria research. Within a year he succeeded in finding two species of a pigmented haemosporidian parasite and a non-pigmented piroplasm in bats near Rome. DIONISI (1899) recognised that the former did not belong to the true malaria parasites and probably possessed a different mode of multiplication; he accordingly placed them in a new genus, *Polychromophilus*. WENYON (1926) removed these parasites to the genus *Plasmodium*, but the elucidation of their full life cycle by MER and GOLDBLUM (1947) and others, enabled the writer (GARNHAM 1953) to restore them to the original genus, in the family Haemoproteidae.

The blood stages of *Polychromophilus*, like those of all parasites in the Haemoproteidae are confined to gametocytes. The youngest forms resemble typical malaria « rings » in erythrocytes; the vacuole disappears with growth, pigment appears and sexually differentiated parasites develop. Their oval shape, though not invariable, is a striking feature. Exflagellation of the microgametocyte is characteristically difficult to elicit in a humid chamber.

The course of sporogony has been briefly described by MER and GOLDBLUM; it takes place in nycteribiid flies (*Penicillidia* spp. in Israel) in which ocysts grow on the midgut, and stumpy sporozoites invade the salivary glands. Further details of the development of the parasites in these flies are given below, and the facility with which these phases are demonstrated is rather striking. The ectoparasitic nature of the vector is of course the explanation; one does not have to search far afield for the transmitter.

Schizogony of the parasite in the vertebrate host is much more elusive, and has only been observed in detail by the Israeli workers. Asexual development apparently occurs in a variety of organs and in different types of cells. The final form of schizogony settles down in the Kupffer cells of the liver where small schizonts grow. Larger bodies have been seen in the spleen, lungs and other organs, and as early as 1907, SCHINGAREV described the transport of unpigmented schizonts by macrophages in the blood. The relationship between these asexual stages is still undefined.

The specific identity of *Polychromophilus* has always been a problem, initiated by DIONISI'S classification of Italian material into *Po.melanipherus* and *Po.murinus*. The former was thought to be allied to the human quartan parasite and the latter to the benign tertian. The morphological distinctions are slight, and for the sake of convenience (and until full details of the life history are discovered) the writer (1966) suggested that the designation *Po.melanipherus* should be restricted to infections in the type host-*Miniopterus schreibersii* (and other species of this genus) while the name *Po. murinus* should be used for infections in all other insectivorous bats.

Records of Polychromophilus in Old World

Europe

Po. melanipherus. The type locality of this parasite is the Roman Campagna and the type host is *Min.schreibersii*.

Infections are common elsewhere in Italy, e.g. Maccarese, Fiumicino. It also occurs in Russia (Vitebsk).

Po.murinus. The type locality of this species is again the Roman Campagna and the host is *Vespertilio murinus.* It is common in these bats and other vespertilionids elsewhere in Italy and Moriggi found a high incidence of the parasite (thought to be mixed with *Po.melanipherus*) in *Rhinolophus ferrumequinum* in Brescia. It has also been reported from Vitebsk in Russia in *Myotis daubentoni*; from near Bournemouth in England in pipistrelles (though subsequent searches 50 years later in this locality by the writer proved negative; the parasite has been recovered however by MOLYNEUX and BAFORT — 1971 — from bats caught in the belfries of churches in Norfolk); from Utrecht in Holland in *Myotis* spp. etc.

Asia

Po.melanipherus. MER and GOLDBLUM made their fundamental discovery of the life history of bat malaria parasites, in bats collected in caves above the PLAIN of SHARON in Israel. Both species of *Polychromophilus* are common in this part of the Middle East.

Po.murinus. As in Israel, the parasite of *Myotis myotis* from Lake Van in Turkey is probably to be referred to this species, although it was identified originally by the writer as *Po.melanipherus.* Similarly, the parasite in *Pipistrellus abramus* from Vietnam was designated subspecies *monosoma* of *Po.melanipherus,* but it is better placed under *Po.murinus.* Another early record came from Portuguese India, where DE MELLO and DE SA (1916) described as *Plasmodium mackiei* a parasite in the erythrocytes of *Myotis mystacinus.* Their crude illustrations and text suggest that the organism underwent schizogony, but probably the divided chromatin represented fragmentation of the nucleus of the microgametocyte. They called attention to the ovoid shape of the gametocytes. If this parasite is considered worthy of subspecific identity, its name would be *Po.murinus mackiei* (DE MELLO and DE SA, 1916). Malaria parasites of *Hipposideros bicolor* (and in *H.armiger* and *H.diadema*) in Malaya were found by EYLES *et al* (1967) and were thought by the writer possibly to be a new species of *Polychromophilus*; later, further examples in other bats in this region were discovered by EWERS.

In an expedition to Kashmir in 1970, Geoffrey de Patourel collected on behalf of the writer, specimens of *Myotis longipes* in the Bhamajo cave near Achabal in Kashmir at an altitude of 1730 m. The temperature inside the cave in October was 18°C. One bat out of 19 collected, showed scanty parasites of a *Polychromophilus* type in the blood films. The gametocytes were all oval and when mature no trace of the erythrocyte was visible. In slightly immature forms, one end of the parasite was straight instead of curved. The pigment granules were particularly heavy, black, and sometimes bar shaped. Occasionally a large vacuole was present in the cytoplasm. The usual male and female morphological distinction was noted. The mean length of the male was 5 u and the breadth 4 u; of the female, the length was 6 u and the breadth 4 u. Sections of the liver, spleen and kidneys of the infected bats were stained and examined but no exoerythrocytic schizonts were detected.

Nycteribiid flies were collected from the infected bat and were fixed in formalin. Sections were made and in two flies sporozoites of a stumpy type were found in the haemocoelomic cavity. The sporozoites in section were straight or curved and measured about 7 u in length.

The most recent investigations of the author on malaria in insectivorous bats were made in an expedition to Sabah in

Borneo and to the Malayan peninsula early in 1972. They are briefly described here as they revealed some interesting details of the biocenose.

The Sabah focus occurs in the Gomenton caves in limestone cliffs rising out of the dense Gomenton Forest Reserve near the bay of Sandakan on the Sulu Sea. The largest cave is reached by a rough track through the heavily leach-infested undergrowth, and in the deepest recesses of the caves are to be found bats and swifts in thousands. The swifts build nests here and are the source of the highly prized constituent of the Chinese bird's nest soup. The bats in the daytime rest on the upper part of the walls where it is completely dark, and they are so clustered together that it seems as if several layers are present. They were easily caught in a mist net, disentangled one by one and put into small cloth bags for later examination. The nycteribiid ectoparasites were then removed, blood films were prepared and organs fixed for histology while the carcases were preserved for identification by J. E. HILL of the British Museum. Two species of bats were collected: 28 were *Rhinolophus creaghi creaghi* and 4 were *Miniopterus australis witkampi*. None of the former were infected with malaria, but all four of the latter had scanty infections of *Polychromophilus melanipherus*. The gametocytes were characterized by their oval shape, dark brown pigment granules, well-marked centrioles and the reddening of the corpuscular margin.

The problem is to find an explanation of the presence of the parasite in one species of bat and its absence in the other. Both occupy precisely the same habitat, both were equally infested with nycteribiid flies, and both genera are common hosts elsewbere.

Insectivorous bats were also examined in Malaya (Selangor) and again a curious difference in the incidence of the parasite was noticed, though the numbers were probably too small to be of much significance. Five specimes of *Myotis* *horsfieldii* were collected from a tunnel 250 m long and 1.5 m high leading from the Ampang Water Reservoir. This sheet of water lies in a basin in thickly forested (diplocarp) hills and the tunnel acts as the overflow. One specimen proved to be infected with a malaria parasite, which differed in a few respects from the Borneo material; the pigment was heavier, the shape was usually subspherical rather than oval, and around the margin of immature gametocytes, numerous projecting filaments were visible. These bear some resemblance to the striking filaments which provide the specific name for the type species of *Nycteria* (*N. medusiformis*); it is possible that this Malayan parasite belongs to the latter genus rather than to *Polychromophilus*.

Another species of *Myotis-M.mystacinus-* was examined for malaria from a totally different habitat near the forest reserve of Bukit Lagong. This bat roosts inside the incompletely furled, central leaf of banana plants, about 3 m. high, and several fit closely into the cavity. Blood films were taken from thirteen bats (including juveniles and infants) but all proved to be negative for malaria. It is possible that the infection is absent because — in contrast to caves (or tunnels) — there is no overcrowding, and in consequence there is no frequent transfer of ectoparasites from one bat to another (compare for instance, on the contrary, the *prevalence* of relapsing fever under conditions of overcrowding in human populations in which their ectoparasite, the louse, proliferates).

The habits of insectivorous bats in Malaysia have been studied in some detail by Lim Boo Liat (1966), but do not yet provide a definitive solution to the problem. He (Muul and Lim, 1970) has however recently invented a completely new method of studying the behaviour of animals in the tropical jungle: instead of single towers or platforms, aerial transects were constructed of aluminium sections through the canopy at a height of over 30 m and 300 m in length. At any point on these sensational walkways, bats and other mammals, insects etc. can be observed under completely natural conditions.

Australasia

Po.melanipherus and *murinus*. The former is probably the better designation of the parasite which McGhee found in *Miniopterus australis* in the New Hebrides. Similarly, this is probably the better name for the parasite in *Min.schreibersii* in Queensland, while the species in other Australian bats *Eptesicus humilis, Nyctophilus bifax* and *Hipposideros semoni* may be identified as *Po.murinus*. The latest record from Australia comes from DEW and MCMILLAN (1971). They found infection rates of up to 83% in *Miniopterus schreibersii* in New South Wales and 42% in *Eptesicus humilis* in Queensland; a single oocyst was found in a nycteribiid fly, but no exoerythrocytic schizonts were detected.

Africa

The identification of African material is complicated by the existence in that continent of *Nycteria* spp. in insectivorous bats. ADAM and LANDAU (1972) studied such parasites near Brazzaville and were able to clarify the systematic position to some extent by the discovery of sporogonic and exoerythrocytic stages. Parasites were found in at least three species of bats of different genera (*Miniopterus, Rhinolophus* and *Triaenops*); in *Minminor* exoerythrocytic schizonts of a *Nycteria* type were found in the lungs and nycteribiid flies collected off these animals were frequently found to be infected with stumpy sporozoites. Bray and Ashford have recently found *Polychromophilus murinus* in *Rhinolophus* in the highlands of Ethiopia and detected rare schizonts in smears of the liver. The gametocytes were round instead of oval and the pigment granules were small; this parasite may well have been *Nycteria.*

Records of Polychromophilus in New World

A fairly large survey of bat parasites in general has been made in the New World, principally on account of Chagas' disease, but also in investigations on yellow fever, leishmaniasis and rabies. Yet the records of malaria parasites are extremely few. *Polychromophilus* has been reported twice from the Amazonian region, and once from California and Texas. There is an unpublished observation by V. Scorza from Venezuela. The first Amazonian case was reported by the DEANES in 1961 from *Glossophaga soricina* (in material collected a decade earlier), and the second by the writer and his colleagues (GARNHAM et al. 1971) from a little further up stream and in a different genus of bats — *Myotis nigricans.* The parasite was given a new name (*Po. deanei*) on the basis of zoogeography.

Dr. SHERWIN WOOD kindly put at the writer's disposal his collection of blood films of bats from Texas and California. In a male *Antrozous pallidus,* collected in 1951 in the San Joaquin Experimental Range, in California, a light infection of *Polychromophilus* was found. The microgmetocytes were subspherical, 8-8.5 u in diameter, pinkish in colour, and several centrioles were present; the pigment was inconspicuous. The macrogametocytes were of similar shape, up to 9 u in diameter, with a small nucleus and no centrioles; abundant pigment in irregular grains was distributed through the cytoplasm. No sign of the erythrocyte were seen. This parasite appears to be longer and more oval than *Po. deanei,* and the pigment in the female is more abundant. Wood found similar parasites in pipistrelles in Texas, but *Myotis* was consistently negative.

Discussion on evolution of Polychromophilus

The essential requirement for zoogeography is the adequacy of the survey, and it is not always appreciated that many maps purporting to illustrate the distribution of an organism merely represent the places where the zoologist has worked. The records mentioned in this paper indicate however the frequency in which *Polychromophilus* is encountered in the Old World and the rarity in the New World. The problem is complicated in the Old World by the occurrence in insectivorous bats of closely related haemosporidian parasites.

The vertebrate hosts are practically all vespertilionids, and *Miniopterus* and *Myotis* continue to be the genera from which most records of infection emanate. The former has a limited number of species and *Min. schreibersii* heads the list of infections; the genus is confined to the Old World and to the warmer parts of the Palaearctic, Ethiopian, Indo-Malaysian and Australian regions. *Myotis* on the other hand is completely cosmopolitan occurring throughout the eastern and western hemispheres to the limit of tree growth. Myotis has many species and several of these in the Old World are commonly infected, and one in the New World.

Nycteribiid flies are faced with few hostile elements, and unlike strebliids easily survive the season of hibernation of the bat. The actual species of vector has been determined in only a few instances. Ectoparasitism of the vespertilionid bats is almost universal. *Basilia* in the New World and *Pencillidia* in the Old World appear to be the chief agents concerned in transmission.

The three components of the infections — bat, nycteribiid and *Polychromophilus* — almost constitute a single entity, and they possibly became linked together from an early age. The rarity of the infection in the Americas presents an interesting problem, and three possibilities may be considered.

1. Parallel evolution of all three components in both the Old and New World seems improbable.

2. Vespertilionid bats are thought to have reached North America via the Behring Straits and to have colonised that Continent, evolving into new genera and species as they travelled to the south. The migrations probably took place in preglacial periods, and it may be supposed that the bats were accompanied by their ecto — and endo — parasites. The extent of the present day distribution of the malaria infection in bats in temperate and tropical climates in the Old World indicates its ability to survive under different conditions.

The complex of bat and its parasites may have reached the New World before continental drift had split Laurasia and the dating of this process, at 70 million or more years ago, (TARLING and TARLING - 1971) makes this theory a possibility.

3. Infected bats may have been transported across the Atlantic Ocean in historical times, either blown across or more probably, taken across in the holds of ships.

The second theory appears to be less valid than the third. If the infected bats had reached the New World at a remote age, the infection, by now, should be widely disseminated; if the introduction were more recent, a rare and focal distribution would be expected. The limitation of the infection to Pará, California and Texas fits in best with the last theory of the arrival of infected bats in perhaps the last thousand years.

GUIMARÃES and D'ANDRETTA (1956) in discussing the evolution of the American genus (*Basilia*) of nycteribiids, make some pertinent observations. They assume that the cradle of *Basilia* was the Old World, and that it was introduced into North America by vespertilionid bats twice during the early Tertiary (c 70 million years ago) and into South

America several times in the Pliocene (c 20 million and on). These time scales do not fit in with migrations in relation to continental drift, but the authors make the significant remarks that *a*) the primary hosts of *Basilia* are vespertilionids, *b*) *Myotis* occupies a central position in this family and *c*) *Myotis nigricans* is the most widely ranging species. And now, it is seen that this species is one of the very few species of bats which is infected with *Polychromophilus* in the Americas.

The early separation of Africa and South America about 100 million years ago is perhaps the explanation of the absence of fruit bats (and their malaria parasites) from the New World, because the Megachiroptera had probably not evolved by that time.

Against the theory of a recent introduction of *Polychromophilus* is the widespread distribution of another blood parasite of bats, *Trypanosoma vespertilionis,* in South America, where according to HOARE (1972), this parasite occurs in *Myotis nigricans* from Argentina in the south, through Brazil to Colombia. The trypanosome is equally common in the Old World, where presumably it originated. However, the life cycle of the trypanosome is less complicated than that of the malaria parasite, and infections of the former may have been more easily established under the new conditions of the Americas. Other cosmopolitan blood parasites exists, of which two common examples are *Trypanosoma lewisi* in rats transmitted by fleas, *Haemoproteus columbae* in pigeons transmitted by *Pseudolynchia,* and *Plasmodium relictum* in passerine birds transmitted by mosquitoes. Owing to the migratory habits of these hosts, the rapid spread of the infection after recent introduction would be a likely event — as is known to have occurred in regard to yellow fever and plague which were brought from Africa to the New World within the last few centuries.

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