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REPORT ON A PRELIMINARY INVESTIGATION INTO MALARIA IN THE SIGUR GHAT, OOTACAMUND.

BY

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By Surgeon-Major Ronald Ross, I.M.S.

The malaria problem consists in finding in external nature the protozoal parasite (*hæmamœba malariæ*, Grassi) which has been demonstrated to be the cause of malarial fevers, but which, while it is easily seen in the human blood, has not yet been identified elsewhere.

Its importance.—The importance of the problem lies in the fact that, until it is solved, accurate methods cannot be devised against a disease which causes a larger amount of mortality, sickness, and, probably, expenditure of public funds than any other tropical disease.

Its difficulty.—Its difficulty is concentrated in the facts that the unknown external form of the parasite may be very different morphologically to the known hæmozoic form, and that, since it is an animal parasite, bacteriological methods are useless for purposes of identification.

Theories as to the x-protozoon.—For the sake

* Read before the South Indian Branch of the British Medical Association.

of brevity, the required external form of the parasite may be called the x-protozoon. That it is capable of living and multiplying freely in external nature under certain conditions is quite clear from the epidemiological relations of the disease, which show that the *contagium vivum* is not generally communicable directly from man to man, but exists *ab initio* in certain localities independently of human occupation. This being the case, the x-protozoon must exist in one of three following conditions:—

(*a*) As a protozoon, free in air, water, soil, rotting vegetation, &c., and capable of an optional parasitism in man. According to this hypothesis, it is most likely to belong to some of the spore-producing orders of the protozoa, namely, the proteomysea, the myxomycetes, the lobosa or the flagellata; most probably the second of these.

(*b*) As a parasite in another animal, by which it is communicated directly or indirectly to man.

(*c*) Both as (*a*) and (*b*), that is, as a free protozoon capable of optional parasitism both in man and in some other animal.

Two questions requiring reply.—In order to attack the problem at the best point, it is advisable to begin with the second of these hypotheses, both because the first hypothesis is extremely difficult to investigate, and because certain recent inductions of various writers (1, 2, 3) strongly suggest that the mosquito harbours the x-protozoon. In attempting to follow up these inductions experimentally, I had reached a point where it was necessary to obtain an answer to two questions, namely:—

(*a*) Are mosquitoes sufficiently numerous in very malarious localities to account for the extreme prevalence of the disease there?

(*b*) Do mosquitoes in such localities harbour a parasite morphologically similar to the *hæmamœba* in man ?

Malaria in the Sigúr Ghât.—The locality chosen by me for a study of these questions was the very malarious Sigúr Ghât or Cañon leading from the Ootacamund to the Mysore plateau. The top of the ghât begins 5 miles from Ootacamund, at a height of over 7,000 feet above the sea-level. From this point, the road descends 7 miles to the bottom of the ghât, which is at a height of 3,000 feet above sea-level, and is on the Mysore plateau. Malaria commences 3 miles down the ghât, at a height of 5,500 feet above sea-level, at Kalhutti, where there is a dâk bungalow and the residence of Mr. Nash, a planter. The rest of the ghât is generally uninhabited in consequence of the malaria; but at distances respectively of $1\frac{1}{2}$ miles and 4 miles beyond the foot of it, that is, on the Mysore plateau, there are two coffee estates, the nearer of which belongs to Mr. Nash, and the further, called Westbury, to Mr. R. Kindersley. The malaria season is during the months of March, April and May and the beginning of June. That the disease exists at Kalhutti is conclusively proved by the fact that the servant of the dâk bungalow and his wife suffered severely from it and had to be replaced, and the new servant, who arrived there during my second visit to the place, was attacked, together with his wife and two children, within ten days. Mr. Nash and some of his employés also suffered. That the disease exists at the bottom of the ghât was shown by the fact that 80 per cent. of the employés and their families on Mr. Nash's and Mr. Kindersley's plantations had enlarged spleens, or were getting

fever at the time of my visits. The species of parasite found by me in the blood of patients was the *summer-autumn* (remittent fever or crescent-bearing species) in all these localties, and the *quartan* in one man at Westbury and in one of my servants.

My visits to Sigúr.—I visited the ghâit on three occasions, namely, on the 22nd April, 5th May and the 1st June; living on the first occasion at Kalhutti, and on subsequent occasions, for a few days each time, at Westbury.

Mosquitoes in the Sigúr Ghât.—With regard to the first question propounded in para. 5, namely, whether mosquitoes were sufficiently plentiful on the ghât to account for the malaria, my results were as follows:—At Kalhutti dâk bungalow, where the servants and their families were attacked, they were very plentiful, being bred in a puddle lying within a few yards of the bungalow and of an irrigation pool, from which the servants took their drinking water. Further down the ghât, however, and for its whole length, I did not succeed in finding a single mosquito grub in any of the pools of the ghât river or its tributaries, nor did I find a single one at Mr. Nash's plantation, where fever was prevalent. At Westbury, however, they were numerous in an old disused well. At both these plantations, drinking water was obtained from pools banked up in the course of running irrigation streams, or from pools naturally formed in the course of nullah streams, in none of which could I find any signs of mosquitoes being present, or at all events so plentiful as to render it likely that the disease was propagated by their agency. I felt myself, therefore, at the point of being obliged to abandon hypothesis; (*b*) namely, the mosquito theory, because it was

clear that, in ordinary drinking water at least, the larvæ of mosquitoes were not nearly plentiful enough to account for the extreme prevalence of the malaria there. I was, however, saved from this conclusion by the discovery that, though larvæ could not readily be found in the ordinary sources of drinking water, the whole jungle abounded with fully-developed mosquitoes to such an extent that it sufficed to sit down in the wood at noon to be surrounded by numbers of a very virulent but small species of the insect.

This species, for which I propose the provisional name of Culex silvestris, does not appear to differ markedly from some species of the ordinary mosquito in anatomical details, though it does so in its habits. While the ordinary species frequent dwellings, the C. silvestris appears to live entirely in jungle and undergrowth, especially in the shady parts, and seldom, except perhaps at nights, enters dwellings. Another remarkable difference in habits is that, while the ordinary species never travels far from pots and puddles containing stagnant water, the silvestris may, in my experience, be seen fully half a mile from any water. Indeed, I found it a matter of considerable difficulty to discover the larvæ at all, and it was only after some search that I met with them in a few scanty puddles at the bottom of nearly dried-up and dark nullahs. The adults must, therefore, be capable of travelling far and living long; and they are certainly also distinguished by their extreme voracity and the strength of their haustellary apparatus which enables them to fill themselves with blood in a few seconds. I fancy also that they are capable of attacking cattle and other animals as well as man.

The presence of the C. silvestris then placed the mosquito theory in a far more favourable position, at least so far as regards the Sigúr Ghât, and it now became obvious that in that locality at least, malarial infection may be produced either by the contact of the insect with the human skin as insisted upon by Bignami (2), or by people drinking water out of jungle pools when thirsty, or perhaps by both means. On the whole, from a consideration of the epidemiological facts, I should be inclined to favour the idea of contact being the mode of infection; and may add that one of my servants who was employed in catching the adult silvestris by allowing them to settle on his legs and arms was attacked five days afterwards by the quartan parasite. The water theory is also possible. It does not follow that infection by water containing mosquito-larvæ is impossible in a locality, because the ordinary drinking water is free from them. Coolies, herdsmen, wayfarers, &c., may, when hot and thirsty, drink occasionally from jungle-puddles and thus contract the disease. Still, after all, puddles containing silvestris' larvæ were so rare in the Sigúr Ghât, and malaria was so common, that this explanation appears to me scarcely to suffice, and I must prefer the contact theory as being the more probable one. Since the presence of a human being in the jungle, at once causes a number of silvestris mosquitoes to attack him on all sides, it is very clear that he may readily be infected by their agency, either by injection of the parasite through the puncture, or by its deposition on the skin in the shape of spores contained in the insect's fæces, which, observation shows, are always discharged in quantity during the act of haustellation.

As an answer then to the first question pro-

pounded I feel inclined to admit as a result of my brief investigation that mosquitoes are plentiful enough in the Sigúr Ghât to account, on the supposition of the mosquito being the alternative host of the parasite, for the presence of malaria in that locality. At the same time I must carefully guard myself from being held to mean that I have found more epidemiological evidence for the mosquito-hypothesis than for the other hypothesis already defined. On the contrary, while I admit from my experiences in the ghât that the mosquito theory is possible, I am inclined to think, as a result of these experiences alone and apart from Manson's inductions based on the evolution of flagellate bodies, that the hypothesis of the x-protozoon living free in the air, water, soil is just as probable, if not more so, than the mosquito theory.

Parasites of mosquitoes in Sigúr.—The other question asked in para. 5 and requiring study in the ghât was whether or not the mosquitoes there are affected with any parasite morphologically similar to the *hæmamœba malariæ* in men. In the intervals between my visits to the ghât, I studied numerous specimens of mosquitoes, namely, of the *silvestris* and of insects from Kulhutti and from the disused well at Westbury. The result was that I found no parasite very similar to the *hæmamœba.* At the same time, however, several new parasitic forms were noted, as well as two forms suspected to be parasitic, and I think it will be useful to describe these briefly in view of the possibility that the *hæmamœba* is dimorphic—in other words that the *x-protozoon* in the mosquito (if it live there at all) is not morphologically similar to the *hæmamœba*—a possibility which will be discussed presently. The following is a com-

plete list of parasites of the mosquito known to me at present:—

(1) The *filaria sanguinis hominis.*

(2) A *nematode* found in the stomach of one *larva* from the Westbury well.

(3) A *fungus,* commonly found by me in the large intestine of mosquitoes.

(4) *Gregarina* (*monocystes*) *culicis.* Probably various species. Found very frequently.

The intercellular and free stages are passed in the stomach of the larva. Towards the end of the larval stage the adult gregarines pass up the *malpighian* tubes, when they become encysted in the pupal stage, each sporocyst containing a number of *pseudo-navicellæ* with a *schneiderian* body and two falciform bodies. These pseudo-navicellæ are ejected through the bowel of the *imago,* partly into the water from which it emerges, and often upon the human skin during haustellation.

(5) Spores of a *protozoal* parasite, probably entirely intracellular, lying along the course of the nerve trunks and around the ganglia in all stages of the insect. The spores, when fully developed in the host, are oval, $5\mu \times 2\mu$ in size, with a clear circular area at one end, which under formalin shows a *nucleolar* (?) point. The spores until scattered lie always in clusters of eight, which represent the bulk of original *cystazoon* and are often surrounded with the shell of the containing (lymph ?) cell. Long-continued efforts to trace backwards the life-history from the spores to the adult and young cystazoa have not led yet to determinate results; but in some cases extremely delicate oval or crecentic bodies have been noted lying in cells similar to those which contain the spores. The fully-developed spores are discharged in an un-

known manner and are found at the bottom of the vessel containing the *larva* where they grow to eight times the size (*sic*—like the spores of *protomyxomyces* (4) and (?) give rise to *amœbulæ*. I doubt whether in this case we have to deal with *sarcosporidia*, or with a body like blood parasites of vertebrates (*danilewskya*, karyolysus, &c., or even *hæmamœba*). It certainly seems to be closely allied to the last. Found in all the Sigúr mosquitoes and also in Ootacamund mosquitoes.

(6) *Coccidia*(?) of the cells of the stomach of the adult mosquitoes, found only in a few specimens. The *coccidia* seen were oval, $8\mu \times 4\mu$ in size, with unsegmented contents, containing a very faint circular spot surrounded by very faint granulation. Under formalin a single segmentation (?) occurred. Each *coccidium* lay by the side of the cell nucleus. Found in the mosquitoes of the disused well at Westbury.

(7) *Sporangia* in the stomach of some newly-hatched adult mosquitoes from the Westbury wells. Under water the sporangia give rise at once to thousands of *flagellulæ* and also (?) *amœbulæ*. The *flagellulæ* are about $8\mu \times 2\mu$ in size with numerous minute black points in their substances and have a single long flagellum. They grow slightly in size and distinctness and attack the tissues of the dead host. I am inclined to think that naturally they descend to the intestines where they adhere and undergo further unknown development, but they may be passed out upon the human skin during haustellation. The stomachs containing these *sporangia* were given to a volunteer, who was attacked with slight fever on the 5th day; but I found no parasites in his blood. Owing to the paucity of specimens, I was not able to

study the original *sporangia,* but they seemed to be without an inclosing cyst-wall and to lie unattached among the food-residues in the stomach of the young imago ; in short, they consisted of free clusters of flagellulæ closely adhering at first but separating under water. At one time I thought that the flagellulæ might be derived from the sporocysts of *englena* which abounded in the well and were swallowed in large numbers by the larvæ.

(8) A doubtful form consisting of irregular masses of *gregarine-like* brown substance amongst the stomach cells, without the shape and movement of gregarines. No sporulation has been noted. Found also in Ootacamund mosquitoes.

(9) Another doubtful form, consisting of very numerous greenish cells containing small or large particles which oscillate violently, or even rotate under water. The cells vary in size up to that of a large tertian *hæmamœba* and seem to be contained in the cells of the *mesoblast.* Found also in Ootacamund mosquitoes.

Numbers 3 and 4 of these parasites were first found by me in Secunderabad mosquitoes, but are seen pretty generally everywhere. Of the rest all except No. 1 have as yet been seen only in Sigúr and Ootacamund mosquitoes; but of course in the limited time at my disposal, I was not nearly able to give them sufficient study. Nos. 4 to 9 inclusive are all *protozoal,* and, I consider, require careful working out in view of anyone of them being the x-protozoon we are in search of, on the supposition of the malaria parasite being dimorphic. It must not be supposed that the mosquito theory becomes untenable because the Sigúr mosquitoes appeared to contain no parasite *morphologically* the same as

the *hæmamœba* in man. In the first place, I may have overlooked such a parasite in consequence of so many of the cells of the mosquito having normally (?) the appearance of containing delicate cystozoa, and in the second place, it seems quite possible that the x-protozoon in the mosquito (if it exists there) may be very dissimilar to the *hæmamœba* in man. For example, R. and L. Pfieffer have advanced certain hypotheses on dimorphism in the *sporozoa*, of which I have read only a criticism by Labbé (5), but which seem certainly to call for extended observation, especially as regards malaria. Thus as regards the *gregarina culicis*, I have been unable as yet to infect larvæ with the pseudo-navicellæ, while I have found the latter unchanged in the lower intestine of larvæ which have swallowed them and have also seen empty pseudo-navicella shells in water; all these facts suggesting some kind of dimorphism in the gregarina. Again, Cunningham (4) has given a number of facts to prove dimorphism, and even a kind of metagenesis in the so-called *amœba coli;* he gives observations (some of which I can confirm from my own studies) to show that the *amœbæ* give rise in the intestines to the *zoospores* (*flagellulæ cercomonads*), whereas in exposed cow-dung they proceed by agmination to the production of a kind of plasmodium, ultimately forming peculiar spores which by another route produce *flagellulæ* and *amœbulæ* again. If such a thing be possible in the case of *amœba coli*, it may also obviously be possible in the case of the *hæmamœba ;* in which case we may expect the x-protozoon, whether in the mosquito or free in nature, to present an appearance very different to the hæmozoic form. Indeed by the latter hypothesis, if it exist free in

nature, we have every reason for supposing such a difference; since the hæmozoic form seems scarcely fitted for independent life outside the blood. In this case it has always seemed to me that a life-history somewhat similar to that of *protomyxomyces* is probable, and that the x-protozoon belongs to the *mycetozod* and lives on the damp walls of houses, damp soil or rotting vegetation. Such a theory, however, affords no explanation of the phenomenon of the evolution of the flagellate body.

In reply then to the second question the Sigúr enquiry failed to give a positive result, but at the same time showed that in the mosquitoes there, several *protozoal* parasites exist, anyone of which may just possibly be a dimorphic form of the malaria parasite. Such a brief study, however, can scarcely be expected to yield determinate results; and indeed it has yielded none such, except the discovery of some new parasites of the mosquito.

A case of fever with short incubation.— Some incidental observations are worth recording. On the 25th April, 76 hours after my reaching the malarious area, I was attacked by rigor and fever, which, however, owing to very large doses of quinine, were not repeated. A single motile and typical intracorpuscular *amœbula* was found by me next day in my blood. The case is interesting on account of the short incubation period, but I failed to trace the source of infection with certainty. I had not been bitten by mosquitoes, nor had I drunk unboiled water; but both myself and Mr. Kindersley, who was also attacked (on the 24th) had drunk tea made very hastily from water obtained either from the old well at Westbury or from a stagnant pool (containing no mosqui-

toes but swarms of a species of *englena*) on the 23rd of that month.

Protozoa in Sigúr water and dew.—I examined many of the pools and water-courses of the locality for *protozoa* and found in excess mostly *englenæ* and sometimes *arcellæ*. Dew on grass contained two species of spores which require further study. I did not look for *myccetozoa*.

Absence of malaria in one spot.—At the bottom of the ghât, there used to be a village, Sigúr, from which the ghât takes its name. This village was abandoned on account of the malaria. During my visits, however, there was a family of Korybahs (a local tribe), consisting of two men, two women and five children, who had lived there for some time, and who, much to my surprise, neither had enlarged spleens, nor were getting fever. I saw them frequently. They drank running river water, while the people in the adjacent plantations, who were so subject to fever, drank water from muddy irrigation pools.

Proposed course for future enquiry.—Further study of the subject should be carried out along two lines, namely (*a*) by endeavouring to infect mosquitoes by means of malarial blood, as formerly proposed by Manson and attempted by me, and (*b*) by pursuing the course adopted in this preliminary investigation. A good opportunity for both purposes appears to be afforded by *kala-azar*, which I understand has now been shown by Surgeon-Captain Rogers to be a form of malaria, and which has long been known to be a transferable disease, a conjunction, which ought to yield a peculiarly favourable field for the study of the malaria problem.