Public health classics

This section looks back to some ground-breaking contributions to public health, reproducing them in their original form and adding a commentary on their significance from a modern-day perspective. Robert E Sinden reviews Ronald Ross's pivotal work on the malaria parasite and comments on the potential for malaria vector research and control.

Malaria, mosquitoes and the legacy of Ronald Ross

Robert E Sinden[a]

ON SOME PECULIAR PIGMENTED CELLS FOUND IN TWO MOSQUITOS FED ON MALARIAL BLOOD.

By Surgeon-Major RONALD ROSS, I.M.S., (with note by Surgeon-Major SMYTH, M.D, I.M.S.)

For the last two years I have been endeavouring to cultivate the parasite of malaria in the mosquito. The method adopted has been to feed mosquitos, bred in bottles from the larva, on patients having crescents in the blood, and then to examine their tissues for parasites similar to the haemamoeba in man. The study is a difficult one, as there is no *a priori* indication of what the derived parasite will be like precisely, nor in what particular species of insect the experiment will be successful, while the investigation requires a thorough knowledge of the minute anatomy of the mosquito. Hitherto the species employed have been mostly brindled and grey varieties of the insect; but though I have been able to find no fewer than six new parasite of the mosquito, namely a nematode, a fungus, a gregarine, a sarcosporidium (?), a coccidium (?), and certain swarm spores in the stomach, besides one or two doubtfully parasitic forms, I have not yet succeeded in tracing any parasite to the ingestion of malarial blood, nor in observing special protozoa in the evacuations due to such digestion.

For the full text of the paper by Ronald Ross (*BMJ* 1897;Dec 18) please see: http://resources.bmj.com/bmj/readers/back-issues-and-archive

In 1895, Ronald Ross was based in Sekunderabad, India, where he embarked on his quest to determine whether mosquitoes transmitted malaria parasites of man. For two years his studies were clouded by observations on what we now know to be insusceptible mosquito species. He nonetheless observed "flagellation" of *Plasmodium* in the bloodmeal of these insects, the true nature of which was revealed by McCallum in 1897.[1] Ross's later work also benefited from the numerous observations on insects infected by other parasites (including helminths, fungi and gregarines) he made in this early phase of his quest for the malaria vector. Eventually in July 1897 he reared 20 adult "brown" mosquitoes from collected larvae. Following identification of a volunteer (Husein Khan) infected with crescents of malignant tertian malaria and the expenditure of 8 annas (one anna per blood-fed mosquito!), Ross embarked on a four-day study of the resultant engorged insects. This "compact" study was written up and submitted for publication.

Imagine today sending an article to a leading medical journal in which you describe observations on novel objects found on the midguts of just two "brown" mosquitoes, obtained from larvae of natural origin, that you had previously fed on a naturally infected patient – with no appropriate controls and no replicates! What hope would it have of getting past the editor and reviewers? Thankfully, Ronald Ross's paper was more fortunate: it was published by the *British Medical Journal* on 18 December 1897.[2] His conclusions were understandably modest. "To sum up: The [putative malarial] cells appear to be very exceptional; they have as yet been found only in a single species of mosquito fed on malarial blood; they seem to grow between the fourth and fifth day; and they contain the characteristic pigment of the parasite of malaria." So begins one of the most influential stories for malaria research and control.

Recognizing the relative simplicity of the research tools available to Ross, the observations made by him and his collaborators using simple brightfield microscopy were exceptional. He had just eight "brown" mosquitoes that had fed on the patient with *P. falciparum* gametocytes in his blood. Four mosquitoes were killed immediately to examine the fabulous process of exflagellation (male gamete production), so critical to the discovery of the bloodstages of the parasite by Laveran seventeen years earlier.[3] One mosquito was dissected on the second day to no advantage and two on the fourth day, of which one had twelve "substantial cells". The descrip-

[a] Division of Cell and Molecular Biology, Faculty of Natural Sciences, Imperial College, London SW7 2AZ, England. Correspondence to Robert E Sinden (e-mail: r.sinden@imperial.ac.uk).

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tion of these cells, the malarial oocysts (formed through the developmental progression: gametocyte-gamete-zygote-ookinete-oocyst) is unmistakeable. The characteristic round/oval shape, the diameter (10–16 microns), the sharp line of the oocyst wall and the nature and distribution of the malarial pigment were reported with precision. The presence of pigment was critical in Ross's eyes, but even this, his defining character, was nonetheless cautiously considered as potentially being a mosquito-derived product of bloodmeal digestion. On the fifth day he dissected the last mosquito and noted 21 cells with the same visual properties, but larger (he estimated the diameter to be about 20 microns). Few today would complain about oocyst intensities and prevalences such as this. There were, however, no controls, such as mosquitoes from the same source fed on a crescent/gametocyte-negative volunteer. In this regard Ross excuses himself, stating "I have not yet succeeded in obtaining any more of the species of mosquito referred to," and felt it was adequate to describe results from other mosquito species (including a genus *Aedes* now known to be refractory to infection by *P. falciparum*) fed on different volunteers. While hardly conforming to the concept of a controlled and replicated study, Ross commendably obtained, and reported fully, a second opinion on the nature of the preparations from Surgeon-Major John Smyth, whose comments are very detailed. The formaldehyde-fixed specimens were then considered to be of such potential importance that they were shipped from Sekunderabad to the United Kingdom to be observed by Manson, Sutton and Thin. Their observations and reviews are also reported in the publication. Manson and Sutton enthusiastically endorsed the views expressed by Ross, and the drawings Manson commissioned unquestionably illustrate oocysts that are either undergoing sporoblast formation before sporozoite budding or possibly degenerating (should the "pebbled" appearance indicate vacuolation). In contrast, Thin sets about a thorough dismemberment of the interpretations of his four colleagues, concluding through logical argument (but with no evidence) that they were describing midgut epithelial cells in which pigment had been phagocytosed from the gut lumen. He then diplomatically apologizes for his unsupportive interpretation!

What can we learn from this seminal publication that is relevant to today's research environment? First, the importance of seizing the opportunity. Second – and related – persistence: Ross recounts that, before the reported successful experiment, work in the preceding two years examining about a thousand brindled, grey and white mosquitoes had failed to reveal any relevant data. Third, the power and importance of careful observation combined with exact and objective recording. Finally, the benefit of sharing data before publication so as to put forward conflicting interpretations of the results. Notwithstanding these commendable attributes, nobody today would have condemned the editor if he had had rejected such a speculative, uncontrolled and unreplicated study.

Irrespective of the perceived inadequacies of the study design, it is difficult to overstate the importance of Ross's paper: the award of a Nobel Prize hardly does justice to the subsequent impact of his conclusions. The biological significance of the paper lies in three areas: basic research; malaria transmission/epidemiology, and the identification of what is perhaps the most vulnerable stage in the parasite life-cycle for effective intervention. The last was very quickly recognized (inevitably in the military–political context) and resulted in the rapid adoption of environmental vector control campaigns (personal protection and house screening to prevent contact with the adult mosquito, and water management to destroy larval breeding sites). These were followed in the 1930s by the introduction of effective insecticides including the "wonder compound" DDT which, together with the new antimalarial drug chloroquine, formed the foundation of the ill-fated but very successful global control campaign of the 1950s and 1960s.

It was immediately clear from these early control campaigns that attacking mosquito vectors of malaria can be one of the most effective ways to reduce the transmission of disease in endemic areas. Research today is identifying an ever-wider range of potential intervention technologies and targets to achieve this objective. In addition to continued refinements of established environmental management and insecticide programmes, a major step forward has been made through the design of effective and environmentally friendly insecticide-treated bednets. New concepts for the reduction of mosquito populations by biological control have been introduced. Variants of this theme include the use of larvivorous predators (*Gambusia* and *Tilapia*), pathogens, e.g. bacteria (*Bacillus thuringiensis israelensis*), fungi (*Beauveria*) and viruses (*Bacculovirus*). Most recently it has been suggested that genetic control of vector populations may be possible. Methods proposed include the introduction of lethal homing endonucleases,[4] the disruption of the olfactory mechanisms that guide the potential vector to the human host,[5] introduction of cytoplasmic incompatibility induced by the endosymbiont *Wolbachia*,[6] or genetic dominant-lethal technologies.[7] Only time and objective study will reveal which, if any, of these approaches will prove to have the appropriate combination of efficacy and practicality to reduce vector populations.

It matters not whether we block the dissemination of *Plasmodium* through endemic populations by killing the vector or the parasite within the vector, the impact upon malaria transmission is comparable. In this regard, the exciting basic studies now under way on the biology of the *Plasmodium* in *Anopheles*, of vector–parasite interactions, and on the mosquito innate immune system have already given us insights into yet more methods by which to modulate parasite dissemination. The question as to whether any mosquito gene that confers refractoriness to *Plasmodium* can be driven into the vector populations remains a challenging and interesting area for investigation. Recognizing the evolutionary forces that have driven the current global distribution and genetic structures of parasite, human and mosquito populations, we must be aware that, just as the fitness cost to the human host in being genetically resistant to *Plasmodium* (e.g. sickle cell anaemia) has driven a balanced polymorphism (stable resistance–gene frequency), the cost to the vector of being refractory to *Plasmodium* may itself be a constraining influence on the future introduction of refractory genes by genetic manipulation technologies.

Notwithstanding the caveats expressed above, intervention in the vector – but targeted directly at the parasite – is one of the more rational approaches to attack parasite populations. Transmission-blocking vaccines are the exemplar

intervention of this type. The reasons for this optimism are founded on the precarious nature of the transmission. Of the thousands of parasites (gametocytes) that might be ingested by the female mosquito, just a handful survive to form the oocysts (as described by Ross in his paper), and this in a small fraction of the vector population. Similarly, the parasite passes through another constraint as it returns, in the form of sporozoites, from the vector to the human host. Although military analogy suggests that such bottlenecks are invariably the best targets for attack, we must further recognize that exposing one's chosen intervention strategy to 10^9 parasite genomes per host (e.g. bloodstage infections) as opposed to 5–50 genomes per host (e.g. the oocyst) is more likely to lead to the rapid selection of resistant mutations. Second, vaccine efficacy is critically influenced by the exposure time of the parasite to the effector mechanism[8]; in the case of current transmission-blocking vaccine targets such as Pv25 and 28[9] this exposure time is 24 hours, as opposed to a few minutes per cycle for vaccines targeting the surface of the bloodstage merozoite. Third, problems that can hinder the development of some bloodstage/sporozoite vaccine include antigenic polymorphism and antigenic diversity, two molecular mechanisms that are logically considered to have evolved in the parasite to overcome the adaptive immune systems of the vertebrate hosts. The mosquito, on current evidence, does not have an adaptive immune system, and it is interesting to note that those molecules expressed de novo on the surface of the malarial ookinete in the mosquito midgut are comparatively non-polymorphic[10,11] and do not undergo antigenic variation, thus rendering them relatively stable global targets for any vaccine. It is encouraging to record the early human trials of pv25 suggest that these vaccines, first described in avian and rodent models, successfully induced 30% blockade of transmission.[9]

An area that for many years has not received the attention it deserves is that of drugs that can target the stages of the parasite responsible for transmission, and which have a realistic possibility of being exposed to effective drug concentrations (if delivered from the human host), i.e. the gametocyte, zygote and ookinete. While it is now known that the gametocyte arrests in the cell cycle with increasing maturity, and is therefore less susceptible than the schizogonic blood stages to many antimetabolites, it is now appreciated that drugs targeting energy metabolism, such as artemesinin and Malarone, can reduce transmission to the vector.[12] Recent proteomic studies further suggest that energy metabolism is upregulated in the ookinete which might render this stage more sensitive to such inhibitors.[13] In view of the high cost of antimalarial drug development, it is a source of constant concern that all potential antimalarials are not routinely screened for their potential to suppress (or indeed enhance!) mosquito infection. Had it been recognized that chloroquine can enhance the infectivity of the drug-insensitive mature gametocytes,[14] it might have been administered differently and might perhaps still be of use today.

Without detracting from the outstanding individual and global efforts made, we have waited far too long to capitalize effectively on the seminal observation made by Ross 110 years ago. The fact that malaria remains as serious a world problem today as it was when Ross and Laveran made their insightful contributions reflects not only the scale and complexity of the interacting populations of *Plasmodium*, mosquitoes and human hosts, but also our financial and political priorities, and perhaps the competitive as opposed to collegiate manner in which research can be supported and conducted. Notwithstanding the most earnest and sustained endeavours of numerous private, national and international agencies, governmental and scientific attitudes must change if the potential for malaria control revealed by the studies of Ross, Manson and their Italian contemporaries is ever to be achieved. The parasite will inexorably evolve, our priorities and attitudes must evolve faster. ■

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