Human Malaria Parasites: Are We Ready for a New Species?

Xin-zhuan Su
Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

(See the article by Sutherland et al, on pages 1544–1550.)

There are 4 well-established human malaria parasites, although a nonhuman primate malaria parasite, *Plasmodium knowlesi*, can also infect humans [1]. *Plasmodium ovale* is one of the human malaria parasites, but it traditionally receives little attention because it causes mild disease and has a relatively low infection rate. In this issue of the *Journal*, an intriguing and provocative article by Sutherland et al [2] raises the possibility that 2 distinct parasite species coexist under the name *P. ovale*.

*P. ovale* was established as a species by Stephens in 1922 after he observed oval-shaped infected erythrocytes in the blood of East African patients [3]. Malaria parasites are small protozoan organisms living within human blood cells and are commonly recognized by microscopic observation of Giemsa-stained smears of blood from patients. Various morphologic and developmental characteristics have been used to distinguish the parasites. These include the size and shape of infected red blood cells and parasite stages, the time required to complete their life cycles, their host preference and specificity, and the disease symptoms they cause. *P. ovale* is morphologically similar to another human malaria parasite, *Plasmodium vivax*, and it can be difficult to tell them apart in blood samples in regions where both parasites are present [4]; however, they can be distinguished on the basis of established differences in morphologic and other characteristics [5, 6].

The introduction of molecular techniques has greatly improved the identification of malaria parasites. Genus- and species-specific polymerase chain reaction methods have been developed to assist parasite identification [7, 8]. *P. ovale* is differentiated from other malaria species by means of species-specific polymerase chain reaction primers designed on the basis of sequences encoding the parasite’s small subunit ribosomal RNA (ssrRNA) and other genes. By applying species-specific amplification methods and DNA sequencing, it has been shown that *P. ovale* parasites worldwide belong to 2 genetic haplotypes: classic and variant [9, 10]. Application of molecular techniques has also led to the discovery of the parasites in many regions not previously known to have *P. ovale*, particularly many Asian countries [6]. In their study, Sutherland and colleagues examined genetic polymorphisms in 55 *P. ovale* isolates obtained from 12 African and 3 Asian countries. They confirmed the presence of complete dimorphism in 5 of 6 loci located on different chromosomes. One isolate from Papua New Guinea had 8 classic residues and 17 variant residues in the ssrRNA gene, but the classic residues appeared to be derived from nucleotide substitutions or multiple recombination events that were unlikely. Sutherland and colleagues wondered why they did not see genetic exchanges between the 2 forms of parasites in their samples. Because the variant and classic forms occurred in sympatry, the genetic differentiation between them cannot be explained by present-day geographic isolation [10], although historical allopatry cannot be ruled out. The investigators found both variant and classic forms in 5 African countries, yet no evidence of inter- or intragenic recombination between the classic and variant forms was observed in their samples. The observations suggest the independent segregation of multigenic haplotypes and the existence of a species barrier, leading to the conclusion that the classic and variant *P. ovale* types are in fact 2 distinct, nonrecombining species. The authors, then, named the species *P. ovale curtisi* (classic type) and *P. ovale wallikeri* (variant type) in honor of 2 outstanding malarologists: Drs Christopher F. Curtis (1939–2008) and David Walliker (1940–2007), respectively.

Further evidence supporting their conclusion is the relatively large genetic dis-
tance between the 2 parasite forms, which is similar to or greater than that seen between the pair *Plasmodium falciparum* and the pair *P. vivax* and *Plasmodium simium* (see Figure 2 in the article). Additionally, the variant-type *P. ovale* has been associated with higher levels of parasitemia in humans [11, 12], suggesting that more dramatic biologic and clinical differences between these 2 parasite types may exist. More studies are needed to investigate whether the 2 types differ with respect to disease manifestations, including the pattern of relapse.

Although evidence from this and other studies strongly suggests that the 2 types of *P. ovale* are 2 distinct species, many questions regarding the actual mechanism of speciation need to be answered before a definitive conclusion can be reached. For example, how have these parasites evolved independently if they can infect the same host species and have coexisted in the same geographic locations? Potential explanations for the observed dimorphism include geographic isolation in the past or the occurrence of 2 distinct host switches from primates to humans separated by sufficient time to allow divergence between lineages. Host switching between nonhuman and human hominids has been suspected for other malaria parasites [13, 14]. As for why the dimorphism persists in the present, several potential explanations other than the suggested species barrier exist. One possibility, as Sutherland and colleagues point out, is that the 2 parasite types may have mutually exclusive mosquito specificities. Some mutations that allow one parasite to adapt to a new mosquito species may explain the differentiation; however, we do not yet have any evidence to support this hypothesis. It is also possible that the observed genetic dimorphism is perpetuated by a low transmission rate and/or clonal infection. Because *P. ovale* can invade only young erythrocytes, infections usually result in a low level of parasitemia [4]. Additionally, it has been shown that infection with *P. ovale* can generate strong and relatively long-lasting protection (partial protection after 6 years) against reinfection, even with heterologous strains [15]. Acquired immunity will prevent a secondary infection and will greatly reduce the chance that an individual would carry parasites with different genotypes and that genetic recombination would occur in a mosquito.

To address these issues, it is important to estimate the transmission rates, the frequencies of natural recombination, and the “neutral” genetic distances of the parasites using more parasite samples. To definitively confirm that a reproductive barrier exists between the 2 *P. ovale* types, one can perform a genetic cross by feeding mosquitoes blood samples that are infected with the variant and classic types. Sporozoites can then be isolated from the mosquito and injected into a chimpanzee (or infected mosquitoes can be allowed to bite a chimpanzee), parasites can be cloned from the chimpanzee’s blood, and DNA from individual parasites can be typed using genetic markers to determine the haplotypes of the cloned parasites. Unfortunately, without a viable in vitro culture technique for growing the parasites, cloning them could be challenging. Alternatively, DNA can be isolated from a single sporozoite and genotyped using multiple genetic markers from different chromosomes to look for recombinant parasites. Regardless of whether we accept Sutherland and colleagues’ claim that 2 species exist on the basis of the genetic evidence presented, their article certainly raises many interesting questions and opens a new field of investigation.

**References**