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Malaria infection and the anthropological evolution

Infecção por Malária e evolução antropológica

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Resumo

Durante a evolução do gênero *Homo* a infecção por malária exerceu papel biológico chave, influenciando até o desenvolvimento antropológico. Os Plasmódios causadores da malária desenvolveram dois tipos de evolução, segundo um ponto de vista biológico, e filogenético. Em particular, os Plasmódios *vivax*, *malariae*, e o ovale poderiam ter coevoluído com a espécie humana ou ter atingido esta durante as fases mais antigas da evolução do gênero *HOMO*. Por outro lado, o Plasmódio *falciparum* teria sido transmitido aos humanos por macacos num período mais recente, provavelmente entre o fim da Idade Mesolítica e o início da Neolítica. Os Autores mostram evidências biomoleculares, tanto diretas como indiretas, de infecção por malária, observada em indivíduos enterrados, reportando ao Mundo Antigo, e trazidas à luz no decorrer de escavações arqueológicas em alguns sítios relevantes Mediterrâneos. Nesta revisão da literatura, os autores organizam evidências modernas. Confirmam o papel da malária ao afetar a evolução das populações no Mediterrâneo. As pessoas que moram nas várias regiões do Mediterrâneo foram progressivamente influenciadas pela malária, ao longo do avanço desta doença. Além disso, as populações afetadas desenvolveram adaptações culturais, alimentares, e de comportamento, contribuindo para uma redução no risco da doença. Entretanto, pode ser que ambas modificações (as biológicas e as dos hábitos) usadas pelos Plasmódios da malária, favoreceram a emergência de grupos de pessoas com maior resistência contra a malária. Todos estes fatores considerados reduziram o impacto demográfico, influenciando de uma maneira favorável o desenvolvimento em geral, e o crescimento da civilização.

Palavras-chave: Malária; Parasitas tipo Plasmódio; Antropologia; Evolução; Humanidade.

Summary

During the evolution of the genus *Homo*, with regard to species *habilis*, *erectus* and *sapiens*, malaria infection played a key biological role, influencing the anthropological development too. Plasmodia causing malaria developed two kinds of evolution, according to a biological and phylogenetical point of view. In particular, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*, would have either coevolved with human mankind (coevolution), or reached human species during the most ancient phases of genus *Homo* evolution. On the other hand, *Plasmodium falciparum* has been transmitted to humans by monkeys in a more recent period, probably between the end of Mesolithic and the beginning of Neolithic age. The authors show both direct and indirect biomolecular evidences of malaria infection, detected in buried subjects, dating to the Ancient World, and brought to light in the course of archeological excavations in some relevant Mediterranean sites. In this literature review the Authors organize present scientific evidences: these confirm the malarial role in affecting the evolution of populations in Mediterranean countries. The people living in several different regions on the Mediterranean Sea sides, the cradle of western civilization, have been progressively influenced by malaria, in the course of the spread of this endemic disease during the last millennia. In addition, populations affected by endemic malaria developed cultural, dietary and behaviour adaptations, contributing to decrease the risk of disease. These habits were not probably fully conscious. Nevertheless it may be thought that both these customs and biological modifications, caused by malarial plasmodia, favoured the emergence of groups of people with a greater resistance against malaria. All these considered factors decreased demographical impact, influencing in a favourable way the general development and growth of civilization.

Keywords: Malaria; Plasmodia Parasites; Anthropology; Evolution; Human Kind.

Introduction

Available paleopathological evidences demonstrate that infectious diseases as a whole became significant as to morbidity and mortality rates for humankind, only after cultural progress which led to development of zootechnics, and subsequently to extension of agricultural techniques (Capasso, 1985, 1988).¹ Development of agriculture and animal breeding characterized by domesticating bovines and cattle and extensive culture same time the “prey” mounts a response, by ameliorating its specific defence mechanisms (i.e. natural immunity defence).

Second event is characterized by so-called mutualistic symbiosis. This last ancestral occurrence has its origin in the prokaryotic organisms’ symbiosis, when these microorganisms started to perform differentiated and well specialized functions in a common cytoplasmic environment.

Third case is representative of parasitic condition. Parasite organism (smaller when compared with its host), has a significant advantage by feeding at host’s expenses, and in this circumstance, should a damage of the host follow, an infectious disease occurs, while should parasited organisms have none, or negligible damages, it becomes a sort of “healthy carrier” of its specific parasite; notably, this last condition may move towards a frank disease if a state of deterioration and/or immunosuppression occurs, to support this pathologic evolution.

When coming back to co evolution, the parasitism concept represents its ideal model. In fact, in this situation the host-parasite dynamics finds its major expression, and each adaptive change of parasite organism has a respective host reaction. This extremely dynamic relation, characterized by an action-reaction paradigm, imposes evolutive changes to all living species interested by this process, and is directly responsible for variations in

1 Agricultural development, dated between years 9,000 and 8,000 b.C. in Middle East allowed humankind to rely on a surplus of food-stuff, and therefore major determinant which limited demographic growth in communities which applied to hunting and vegetable, harvesting was not reduced. Otherwise, specialized jobs like metal manufacturing (which plays a fundamental role in weapons production), became prominent. Subsequently, history showed emerging of an aristocracy devoted to military practices, paralleling an intellectual class which addressed its efforts towards religious-sciamanic rituals performance. In appropriate geographical and environmental contexts, human communities have the opportunity to address their main activities towards merchant arts, and subsequently evolved their commercial trade by sailing & boarding Mediterranean coasts. Phoenicians represented earlier, entire Mediterranean sea great sailors and had the merit to develop a first alphabet already during Bronze age. Approximately at the same time, Babilonian inhabitants discovered sexagesimal calculation system.

heterochrony, typical of ontogenesis (Capasso and Di Tota, 1995).

The host who suffers from a “non-compensable” damage caused by parasite-infectious agent is destined to succumb; on the other hand, eventual parasite organisms which cause too relevant damage to their specific host have no space in evolutionary selection of species. “Ideal” relationship occurs when variations of one partner represent a selective pressure which induces emerging of a host population which is progressively less reactive, and a parallel population progressively less virulent and less aggressive of parasite organisms.

When parasites are species-specific, an epidemiological balance becomes evident between host and parasite (evolving toward a parasitism without damage/disease, or a symbiotic relationship)². However, it is also possible that virulence pathogenicity features of single parasites, i.e. their capacity to induce damage to specific hosts may be increased by repeated passages of same organisms in hosts belonging to different species. All parasites which have a proportionally broader host-range spectrum are favoured in eluding the selective mechanisms of epidemiological balance, and may still cause severe disease forms, despite their “old” parasite-host relationship. From this point of view, Influenza virus model is very relevant and up-to-date.

As early as in year 1963, Cockburn summarized these concepts as follows: “Infectious diseases affecting modern human kind are the result of all relationship that our species had in the past, and still has, with organisms which acted and still act as parasites” (Cockburn, 1963). As a whole, biological history of human infectious diseases had its development thanks to two different types of relationship: in the first case, a dual relationship between man and parasites (including parasites “specific of humans”), lead to a parallel, true co evolution of both “partners”. Otherwise, this relationship is extended

to hosts other than humans; this last issue predominates when relevant parasite has a spontaneous, extensive host range (Capasso and Di Tota, 1995).

These two different modes of relationship were affected by temporal changes, so that human-parasite dynamics has driven in a significant way mankind’s evolution. On this preliminary basis, we can imagine, by hypothesis, that infectious diseases have represented a very relevant way of selective pressure in different environmental ecosystems, on a worldwide basis, and may have conditioned ontogenetic mechanisms too, possibly modifying the evolutive potential of single species and organisms (Capasso and Di Tota, 1995).

Through an accurate revision of existing literature evidences, we can make an attempt to verify whether presently available data may add significantly to establish what was (and what is) the biological role played by a diffuse infectious disease-like malaria, which represents one of the most relevant infectious disorders registered during long-term evolution of genus *Homo*, considering three species (*Homo habilis*, *Homo erectus*, and *Homo sapiens*).

Infections by Malaria Plasmodia in Nature

Malaria plasmodia descend from ancient endocellular parasites of enteric tract of a common ancestor of reptiles, amphibians, and birds, all of them parasited by some form of *Plasmodium* spp. These endocellular parasites take origin from ancestral, free forms, which occasionally remained trapped by phagocytes of multicellular organisms, where they were able to survive³. Subsequently, ancestors of plasmodia acquired ability to transfer themselves from intestinal cells towards liver cells (through portal circulation). When colonizing a liver tissue, plasmodia might complete their vital cycles, and may spread into systemic circulation of their host. Parasite cycle was re-

2 From a theoretical point of view, the older is the first contact with infectious agents, the milder is expected to be “modern” disease, since in these last conditions a very long time is elapsed, to allow development of a balance between host and its potential parasite.

3 As known, ability to survive for a variable time into phagocyte cells of immune system has been demonstrated for different bacterial species (i.e. meningococci, gonococci, mycobacteria) (Cockburn, 1963).

activated when a hematophagous arthropod insect,⁴ by stinging its relevant host, became able to transfer this infection to another individual, which therefore became parasited. On the ground of paleontological evidences, we can suppose that plasmodia, already present for a long time before the appearance of first vertebrate beings, circulated among primates, transmitted by mosquitoes of genus *Culex*. In New World, this situation remained unmodified for a long time, during mean and late Tertiary ages, until the genus *Anopheles* appeared in the Oligocene period (but only in the Old World), and became plasmodia's vector infection among Primates. This event has been dated at the end of late Tertiary age. After this time, during the late Pleistocene age, humans finally appeared in African continent, and immediately it became involved by epidemiological chains of different plasmodial infections. As a consequence, this assumption explains why mosquitoes of genus *Culex* remained selected vectors for birds and mammals from the Cenozoic era, while primates and subsequently early hominids were simply involved in pre-existing epidemiological chains (Capasso, 1985; Capasso and Di Tota, 1995).

Comparative genotypic analysis of plasmodia, and in particular their comparison of DNA contained in the 18S ribosomal gene, suggest that the divergence of *Plasmodium falciparum* from its more close plasmodial species, the *Plasmodium. Reichenowi*, which parasites chimpanzees, occurred approximately five to ten million years ago, and in this last case a co evolution has occurred (Sallares et al., 2004). As opposed to evolution of *Plasmodium falciparum* into three other species which cause disease in humans (*Plasmodium. vivax*, *Plasmodium malariae*, and *Plasmodium ovale*), passage of these parasites could happen through a collateral pathway, and possibly directly from other primates to humans (Figure 1).

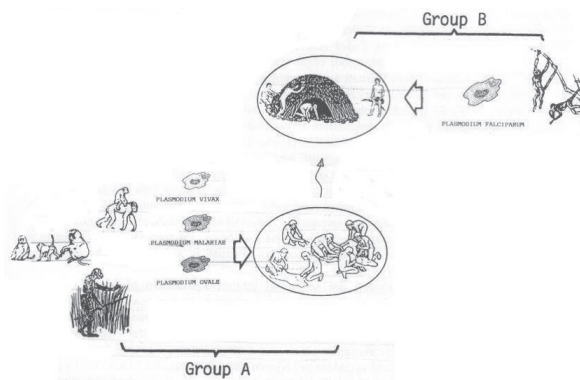


Figure 1 - A graphic representation of biological and phylogenetic evolution of *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale* (group A parasites), and more recent pathway performed by *Plasmodium falciparum* (group B). First three mentioned *Plasmodia* completed their coevolution in parallel with that of mankind or, alternatively, human encountered these protozoa during most ancient phases of evolution (so-called structural parasites). On the other hand, *Plasmodium falciparum* has been met during proportionally more recent times, and it has been acquired after spread among monkeys. Figure is modified from cited paper, by L. Capasso (1998).

An intense debate emerged with regard to most recent molecular evolution of *Plasmodium falciparum*. A controversial statement points out that *Plasmodium falciparum* populations presently in third millennium might descend from a common ancestor, a still surviving species - alive a few thousands of years ago - during the Neolithic period. Otherwise, different investigations underline that *Plasmodium falciparum* maintained a dimension of a real population, also incorporating an elevated degree of genetic diversity, during hundred thousand years of evolution. These apparently divergent statements may result from the fact that researches based their studies on different databases, and non-coincident methods were applied for molecular biology investigations; moreover, problems linked to a different dating affected these last examinations. In a recent and more extensive report, a synthesis of both points of view has been informed, and the Au-

4 Fossilized mosquitoes have been detected in the Eocene limestone layers of *Green River Formation*, in Colorado, USA (Grande, 1980); in majority of cases, insects were represented by *Culex* species (Cockrell, 1921), responsible for transmission of infection to birds, while, as already known, transmission of plasmodia among primates is due to mosquito belonging to genus *Anopheles*. Fossilized remnants of this last genus were retrieved in the Old World only. Luigi Capasso supposes that genus *Culex*, which appeared during early Tertiary age, guaranteed spread of plasmodia in this particular time period. Only later, during mean Tertiary age, circulation of plasmodia will be ensured by mosquitoes of genus *Anopheles* (Capasso and Di Tota, 1995).

thors concluded that most recent, common ancestor of *Plasmodium falciparum* may be dated at around one hundred thousand years ago; furthermore, a major expansion of this last plasmodial species should have occurred during last 10,000 years (Joy et al., 2003).

On the ground of molecular biology data, this infection is recognized as very old in nature. As mentioned by Mario Coluzzi, its evolution into a human parasite may be strictly linked to progression of its more effective vectors in African continent tropical regions, such as expansion of mosquitoes group belonging to a species called *Anopheles gambiae*. Since ecotype living in the forest has been established as the oldest one, we may therefore suggest that anthropophilic behaviour probably started in a limited forest environment, where mosquitoes have a small number of great-size animals at disposal, and may have a greater tendency to affect humans (Coluzzi et al., 2002). Early hominids were thought to live in an environment characterized by rain forests as opposed to open, grassy grounds. Presently, these human ancestors are thought to be already present in Western and Eastern Africa around six million years ago (Mercader, 2002)⁵. Results of these multidisciplinary researches lead to conclude that in forest ecotype of tropical Africa, first contact between *Anopheles gambiae* and *Homo sapiens* occurred, and this event was not necessarily so recent (i.e. dating at Holocene period), but may be much older, and may have involved earlier human ancestors.

If bio molecular studies of DNA of *Plasmodium falciparum* suggest that this last parasite is much older, role played by *Anopheles gambiae* remains essential in order to establish how and when malaria spread had its diffusion, since increased growth of *Plasmodium falciparum* in the population of the Holocene era was probably supported by evolution of other ecotypes of *Anopheles gambiae*, different from original ecotype which had its habitat in rain forest (Sallares et al., 2004).

Demographic expansion

To conclude, the common ancestor that had a development around one hundred thousand years ago is probably related to first modern humans' demographic expansion. However, role of vector *Anopheles gambiae* was determinant in order to make its diffusion epidemic, and conditioning by this way the development of humanity itself. Figure 2 depicts a sample of mosquito, included and preserved in an amber fragment.

On the other hand, Luigi Capasso attributes a significant role to this mosquito species. This last anthropophilic insect may have been a relevant vector of plasmodia to our ancestors, since it had its endemic area along the East African coast, between late Pliocene and early Pleistocene (Bradley, 1993), concurrently with presence of genus *Homo's* first members.

An interesting, but still open question, regards which species of mosquito played a key role in transmission of malaria plasmodia in the New World, before "exploration" carried out by Christopher Columbus. On the above-mentioned dissertation ground, we may adopt the hypothesis of a separated evolution of mosquitoes present in LD, and in New World respectively, since it parallels separated evolution of primates in these two different geographical contexts. A different species' "remixing" of both mosquitoes and plasmodia occurred in a late period, i.e. when Euro-Asiatic and American continents became closer, thanks to Bering Strait practicability, consequent transfer of humans to American continent⁶; consequently, earlier, separated development of both vectors and plasmodia had its first opportunity to blend, when New World became finally inhabited.

As known, plasmodia extensively affect primates, and a number of simian species suffering from malaria has been identified: its clinical picture is characterized by fever and splenomegaly, but an asymptomatic course is not infrequent. For instance,

5 A recent archaeological research points out that human populations of hunters-harvesters were continuatively present in rain forest environment for a long period of time (Mercader, 2002).

6 Humans are known to have reached Americas through Bering Strait around 30,000 years ago; however, adequate findings able to document their presence are dated at 15,000 years ago.

an African chimpanzee may be infected by *Plasmodium malariae*, and this infection may be subsequently transferred to humans, while American primates are parasited by *Plasmodium brasilianum*, and also this species may infect humans causing a febrile illness similar to quartan fever (Capasso, 1998). Human malaria infection is usually well tolerated by primates (in particular, gorillas and chimpanzees), and also *Plasmodium falciparum* of greater monkeys induces a more benign disease, compared with a human one. These differences also indicate that this last *Plasmodium* species had contacts with non-human primates for a very long time, and this “old” contact led to a partial resistance to this specific infection.

During their evolutionary course epidemiological balance problem between *Plasmodium falciparum* and human kind has resolved (from an evolutionistic point of view), after development of a genetic resistance based on establishment and persistence of a polymorphism of genes which encode for protein⁷.

Peculiar features of malaria caused by *Plasmodium falciparum* in humans, which are extensively different from those observed in malaria disease induced by *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*, may suggest that this most extensive contact with *Plasmodium falciparum* occurred in a proportionally more recent time, compared with other malaria forms⁸. It's a general opinion that massive human malaria infection from animal reservoirs, occurred in Africa after introduction of agriculture, and progressive diffusion of a sedentary lifestyle (Grmek, 1985). Probably, massive deforestation carried out in Eastern Africa to acquire cultivable soil, greatly supported mosquito proliferation. Mohe, ensured by stagnant waters, which progressively took space around primitive

villages (Capasso and Di Tota, 1995; Brothwell, 1967). Subsequent step: modified habits of some mosquito species, which ceased their nutrition of primate blood, evolving towards nutrition of human blood. This last occurrence was crucial point for the disappearance of *Plasmodium falciparum* as a major parasite of wild primates⁹, to become one of the most important elements of natural selection of human kind (Capasso and Di Tota, 1995).

In our previous discussion, we examined the possibility that New World has been (infested by *Plasmodium falciparum*) malaria only after discovery of Columbus (which occurred in 1492), since possible, prior contacts regard low populated regions of North America, were probably sporadic in frequency, and probably not sufficient to establish a well recognized danger of malaria spread. However, an unanswered question has been posed, of relevant scientific interest: on which basis it may be stated that malaria was a disease unequivocally limited to Old World, until 1492? In order to try an answer to this intriguing question, both paleopathology and population genetics give us some relevant evidences. As known, malaria (especially in its chronic-relapsing form) induces some characteristic skeleton stigmas, sometimes absent, but when characteristically recognized, may lead to confirm its role in these skeleton deformities. These bones of malaria, named “porotic hyperostosis” from their morphologic features, are common to other conditions of chronic anemia, due to vitamin and/or iron deficiencies. Congenital anemias (i.e. thalassemia, cell anemia, constitutional anemia), may also lead to a picture of “porotic hyperostosis”. Furthermore, also extensive and prolonged intestinal parasite diseases may induce a syndrome, which in its more severe forms may support pathological damages

7 Both sickle-cell anemia and thalassemia, with respective genetic-encoded hemoglobin variants, are the cause of extremely unfavourable phenotypic conditions, with respect to plasmodia survival inside red blood cells of heterozygous subjects. The selective pressure exerted by malaria endemicity in some regions of sub-saharan Africa, is responsible for a proportionally elevated incidence of heterozygote individuals, which bear a “sickle-cell trait” in around general population's 40% (Lambrech, 1967).

8 When considering these three different malaria subtypes, a real epidemiological balance is highly suspected, since a “parallel coevolution” occurred, while in the case of *Plasmodium falciparum* result depended on a forced, elevated selective pressure (a situation which does not occur in great African primates: they usually show a very mild disease course).

9 Primates, after agriculture introduction and consequent increase of human aggregation, ceased to represent malaria, since human are infected only sporadically with simian plasmodia (i.e. *Plasmodium brasilianum*, *Plasmodium simium*, *Plasmodium cynomolgi*) (Capasso and Di Tota, 1995).

not different from those of “porotic hyperostosis” encountered in human malaria (Capasso and Di Tota, 1995). However, even when taking into account these limitations, the paleopathologic investigation on human remnants may address (together with other proofs), to establish eventual responsibility of *Plasmodium falciparum* in supporting this pathological condition. Given that genetic-based hemoglobinopathies represent a reliable marker of population involvement, should *Plasmodium falciparum* be endemic for a long time, among American Indian populations¹⁰ these alterations were detected only when genetic exchanges with subjects of African or European origin occurred, whereas they were absent when these population interactions were not recorded in the past (Capasso and Di Tota, 1995). DNA’s extraction sequences of *Plasmodium falciparum* from bone specimens of human skeletons which, due to a concurrent “porotic hyperostosis” may have been affected by malaria, could contribute to solve this persisting controversial issue.

Starting from last decade of the twentieth century, molecular biology techniques allowed to reliably establish the existence of an ancient *Plasmodium falciparum* infection. The laboratory assay named “ParasightTM-F”, which is able to retrieve antigen of the -rich protein 2 (the so-called protein PfHRP-2) descending from *Plasmodium falciparum* trophozoites, may help in malaria antigen specific identification of *Plasmodium falciparum*. In fact, tissue samples collected from naturally dried human mummies coming from Egypt and Nubia (dating around 5,200 and 1,450 years ago), were tested positive at the PfHRP-2 protein search, while tissue samples coming from subjects detected in the “Camaronas” site, located in the Atacama desert (South America-Chile), and dating around the year 1,000 b.C., were proved negative at the same laboratory search (Miller et al., 1994).

As we may synthetically assess, although bio molecular investigation has reached very advanced

scientific standards, assessment regarding ancient history of malaria infection is still subject to some uncertainty. In order to reach our objectives, it becomes easier to trace back ancient figures starting from present knowledge, linking scientific evidences coming from molecular biology, with both available historical-medical evidences, and archaeological materials and related studies (including human remnants or archaeological findings found in sites explored during proportionally recent years).

When limiting our considerations to the European continent, we may reasonably suppose that during glacial periods of Quaternary era (around two million years ago), until Mesolithic era, malaria remained absent from our continent, since it is a temperature-dependent disease, and mosquitoes were not able to survive in this climatic environment. Subsequently, quotations regarding intermittent fevers reported by ancient literature (Hippocratic reports), dating at fourth and fifth century b.C., some old Indian documents (of difficult dating), and Chinese literature (dating around the first millennium b.C.), do not leave doubts that the benign tertian malaria (due to *Plasmodium vivax*) and the quartan fever (caused by *Plasmodium malariae*), were already endemic in Greece, India, and China. We may therefore reasonably think that these benign malaria forms reached Greece between the end of the last glaciation era, probably at the year 500 b.C., although no serious documents exist (Salares et al., 2004).

While there is a general agreement in retaining origin of malignant tertian malaria (due to *Plasmodium falciparum*) located in tropical Africa (Conway et al., 2000), Roman historical Celsus (who lived in the first half of the first century A.D.),¹¹ made the first differential diagnosis between clinical course of *Plasmodium falciparum*-related fever, and the more benign *Plasmodium vivax*-related disease. Awareness that already existed during the Roman age, showing a substantial difference between two

10 In the Americas, the “porotic hyperostosis”, which has been found in an elevated number of pre-Colombian human remnants, is probably related anemias due to vitamin or iron deficiency, which were frequent after the extension of agriculture, and the diffusion of a corn-based dietary habit (Behar, 1968; El-Najjar, 1976). Other possible causes may be searched among the frequent chronic of the gut, and the endemic presence of bartonellosis in Southern America (Miller et al., 1994).

11 Celsus. *De Medicina*, III. 3.2.

main forms of malaria fever that had been recognized, comes from documents of ancient physician Celsus, as reported around year 100 b.C. (Archigene of Apamea)¹². It was certain that physicians of ancient Roman world were already aware that patients suffering from a malignant semi-tertian fever had a worse course in autumn, but this complication was not so frequent in older ages. This consideration suggests that Italy included a novel disease, clearly identified for the first time in this historical period, and different clinical variants of malaria were endemic in central and southern Italy (Sallares et al., 2004).

Mirko Grmek, one of the most important contemporary experts in Medicine history, on the ground of literature evidences, suggests that only malaria fevers interested the Athenian army (which besieged the city of Syracuse, Sicily, during the Peloponnesian war) (Grmek, 1989). On the ground of this observation taken from literature, and re-enforced by knowledge of already existing marshes around the city of Syracuse, we can reasonably state that 2,500 years ago malaria was already endemic in Sicily. Numerous colonies founded by Greek populations in Southern Italy, between years 800 and 600 b.C., during subsequent decades were severely affected by malaria epidemics: in particular, the historical city of Paestum, where extraordinary temples and monuments were built between the sixth and the fifth centuries b.C., experienced a rapid decline just because of malaria epidemics (Sallares et al., 2004). After an earlier historical phase, which involved centuries preceding 400 b.C. (limited to Sicily and Southern Italy in its coast sites), malaria was diffused in Italy (in a South-North direction, toward central-western regions), when the so-called second stage of malaria spread had its origin (Sallares et al., 2004). During an initial time period, until around 400 b.C., Etruscan cities of Maremma region in Tuscany (Italy), had their period of expansion, as demonstrated by relevant archaeological findings, like vases imported by Greece. Moreover, continued

and closed contacts with emerging Roman potency characterizing this period (in fact, the last three Roman kings were all of Etruscan origin). In these locations, it is possible that malaria spread along Tuscany coasts during third and second centuries. Cato the Senior refers to presence of malaria fever in Graviscae, port of the Etruscan city of Tarquinia, around the year 150 b.C. (Sallares, 2002). Probably plasmodia and their vectors reached the northern Latium coasts (central-western Italy), facilitated by sea transfers from Sardinia, Sicily, or directly from Northern Africa. Please don't forget that, during preceding centuries, citizens of Syracuse and greeks, after defeating Etruscans in the naval battle of Cuma (474 b.C.), achieved control of Thyrrhenian sea, exerting their check on maritime routes and commercial exchanges with regions where malaria had already been an endemic disease, for a long time before. During late second century b.C., in the city of Rome, Asclepiade of Bitinia described severe, intermittent febrile illnesses, sometimes complicated by central nervous system involvement, and Galen quotes this information in his writings, three centuries after.¹³ Rome was certainly surrounded by territories infested by malaria parasites also during imperial centuries, and when considering also general decline following Roman Empire dissolution, even more during subsequent centuries. Vatican correspondence archive studies demonstrated that pilgrims coming from Northern Europe (around the eighth century b.C.) preferred to make their way to Rome during winter season, in order to avoid "the bad air" which polluted environment during summer period of the year (Mc Cormick, 2001). This way followed by malaria parasites towards North-Eastern Italy lasted for a while. Around 2,000 years ago the Po river delta region, not far from the city of Ravenna (the Western Roman Empire's last capital), was reported to be free from malaria. This last information comes from ancient Roman writers Vitruvius and Strabone,¹⁴ and sounded strange at that time, since surrounding regions were already marshy. Accord-

12 Quoted from Aetius of Amida (former Mesopotamian region). Aetius di Amida. *Libri medicinales*, VI.3.

13 Asclepiades ap. Caelius Aurelianus, *De Morbis acutis*, II. 63-4; Galen, VII. 435 and XVIII. 121-2, *Opera omnia* (Kuhn C), Leipzig, Germany, C. Cnobloch 1821-33

14 Vitruvius, *De Architectura*, I.4.11-1; Strabo, *Geographia*, V.1.7.213C.

ing to Sidonio Apollinare,¹⁵ should mosquitoes be present, they were probably not sufficiently effective in transmitting malaria parasites: in fact, malaria became endemic in these regions only during Middle Ages, when an anthropophilic mosquito (*Anopheles sacharovi*), appeared and became permanent along North-Eastern Italian coasts. In these regions, malaria epidemic had a very slow spread, and according to Sallares and co-workers, it happened in a range of 1,500 years (Sallares et al., 2004). Since both *Plasmodium vivax* and *Plasmodium malariae* were present in Mediterranean, Europe before the appearance of *Plasmodium falciparum*, a selection and an extensive spread of an effective vector was needed to support their stable circulation. Specification of a mosquito belonging to *Anopheles gambiae* complex made probably possible a step towards an endemic behaviour of malaria (Coluzzi, 1999). In fact, only when an efficient vector, capable to survive at cold temperatures of North-Eastern Italy, was selected, *Plasmodium falciparum* and two other *Plasmodium* species had possibility to take root in geographic areas which were more favourable to their features (often following field abandonment typical of Middle Ages period¹⁶).

Direct Bio Molecular Evidences of Presence of Malaria in Ancient World

Very recently, in the year 2008, a study which presented very reliable data of identification of ancient malaria DNA (DNA) has been published, together with evidence of *Plasmodium falciparum* in Egyptian around 4,000 years ago (Nerlich et al., 2008). The Authors analyzed 91 bone tissue samples of a parallel number of mummies and skeletons retrieved in all different Egyptian sites¹⁷.

All specimens have been tested for *Plasmodium* spp. DNA with a polymerase chain reaction (PCR), technique which is based on use of DNA of 18S ribosomal subunits, in order to identify unambiguously eventual presence of an ancient malaria infection. Moreover, in two human remnants coming from two different New Kingdom sites, positivity for malaria infection was found, with specimens matching with osteopathological signs of chronic anemia (Nerlich et al., 2008). The same assay performed on tissue samples obtained from remnants belonging to earlier time periods, proved negative for malaria infection (Nerlich et al., 2008).

On the ground of obtained results, the authors critically notice that studies performed during recent years probably overestimated real malaria infection amount in ancient Egyptian world (Miller et al., 1994). These studies employed so-called antigen-2 (a protein rich of istidin), which was detected in over 40% of tested specimens, and in 92% of specimens of human remnants, where signs of "porotic hyperostosis" were appreciable (Miller et al., 1994). Even taking into account this criticism, the same authors concluded that *Plasmodium falciparum* infection played a significant role in conditioning limited life expectancy of ancient Egyptian populations, on the basis of molecular biology examinations conducted with PCR techniques (Nerlich et al., 2008).

As mentioned in the above paragraph, if existing evidences document show, progressive spread of malaria from Southern to North-Eastern Italy, multiple scientific evidences cumulated during last years confirm this last theory. Between years 1988 and 1992, an archaeological site has been discovered in Lugnano (in Teverina), a small town located in Tiber river valley¹⁸ (central Italy), located 70 miles far from Rome. This site includes an extraordinarily large

15 Sidonius Apollinaris, *Epistulae*, 1.8.2.

16 In other terms, as Sallares and coworkers wrote, "...all three species of *Plasmodium* were ready to move together, simultaneously toward novel regions, but they had to wait for appropriate vector mosquitoes in order to start moving" (Sallares et al., 2004).

17 The most ancient site is pre- and proto-dinastical site of Abido (a High Egyptian town) (dating around 3,500-2,800 years b.C.), second site is represented by a grave in West Thebes, of Mean Kingdom time (2,050-1,650 years b.C.), while third site refers to different tomb complexes, also localized at West Thebes, which have been built between mean and novel Kingdom, and until later periods (from 2,050 up to 500 b.C.).

18 Archaeological site is located at 185 meters above the sea in a hill area, 3,5 km far from river (Soren et al., 1995). River valleys represented the earliest malaric areas in Italy, at end of Western Roman empire, since river beds were not maintained, and when overflows occurred, very favourable sites for mosquito replication appeared, especially when flow waters retired leaving small or large ponds. In specific case of Lugnano, this small town is located a few kilometers far from the Orte river's immission into the Tiber river, so that this particular geographical area was at greater risk for malaria spread.

children cemetery of Roman Italy¹⁹, dating at around 450 b.C. (Soren et al, 1995; Soren and Soren, 1999). Archaeological evidences brought the Authors to conclude that deaths were secondary for an epidemic disease which acted in a proportionally short time period, probably during Summer²⁰ (season burdened by highest risk of malaria diffusion), as established on the ground of analysis of remnants of plants and seeds retrieved in the graves (Sallares, 2002).

Among 47 human remnants retrieved, 22 were represented by premature children²¹, and also majority of other dead bodies were neonates. As anticipated, both environmental features and characteristics of retrieval induced researchers to establish that neonatal deaths were due to a severe malaria. This statement has been examined by experts like Mario Coluzzi, who confirmed this opinion, on the basis of environmental, biological, and entomological considerations (Sallares, 2004). Molecular biology assays were also performed, through extraction of DNA genome of *Plasmodium falciparum* from bone specimens. In one case (i.e. number 36) extraction of plasmodial ribosomal DNA was attained by amplifying two different specimens. This last case regards a female child who was aged 2-3 years, at time of death (Abbott, 2001). Feasibility of DNA amplification of *Plasmodium falciparum* from 1,500-years-old skeleton suggests a massive malaria infection²², and not an asymptomatic course due to a low parasite load. This last result of relevant scientific value led to conclude that this death was caused by malaria, thus demonstrating that Tiber river valley was a place of epidemic malaria around the year 450 back. (Sallares, 2004; Abbott, 2001). The Authors finally observe that presence *Plasmodium falciparum* in

central Italy, probably associated with its main vector, i.e. *Anopheles labranchiae* (still present in Tiber river valley during World War II), significantly might increase risk of developing malaria, (of course this is valid for resident population) (Sallares, 2002). Since these Italian regions were already populated, by that time, epidemic events of malaria were expected.

Indirect Genetic Evidences of Malaria Spread in Ancient World

Genetic population studies, performed during past 25 years, it gave us elements to understand which was the era of initial selective pressure operated by malaria on Mediterranean populations. Most recent data confirmed previous suspicions, i.e., this pressure started in proportionally recent times. This observation is acceptable since during previous times climate was too cold (at least in Europe, and during glacial ages), to allow malaria spread.

When underlying that most ancient osteologic evidence of thalassemia, characterized by typical “porotic hyperostosis”, has been first detected in a site close to the village of Atlit Yam, quite far from the Israeli coastal line, and dating around 10,000 years ago (Hershkovitz et al., 1991), population genetics allowed to find a lack of diversity which is usually associated with the largest number of mutations conferring resistance to malaria in modern Mediterranean populations. These evidences suggest that these changes probably represented an evolution started from a time not beyond 4,200 years ago (Tadmouri et al, 2001). All major determinants of inherited genetic resistance were already present in Mediterranean basin at time of Roman

19 In these archaeological cemeteries, findings referring to spells were found, like the presence of skeletons of dog puppies, which maybe served as a weapon against devils, which were thought to be responsible for children's lethal disease. The term “abracadabra”, of common use in magic rituals, was originally employed as a spell against tertian fever, as reported by writer Quinto Sereno (1950). We also remind that children infrequently received a burial during Roman time. It makes these retrievals of extremely relevant interest, since they allow to advance paleopathological, anthropological, and archaeological questions.

20 Some accurate literature evidences exist regarding reknown unhealthiness of these areas. During the Summer of the year 467 Sidonio Apollinare run along Italy from Ravenna to Rome, to encounter Emperor Antemio. During his journey, he just reached the malaric regions of ancient Umbria and Tuscany, leaving a striking infamous effects evidence of “poisonous miasmas”, severe fever, and insatiable thirst: all clear witnesses of malaria disease.

21 Malaria predisposes not only to elevated mortality rates, but also a striking predisposition to abortion among pregnant women, causing an intrauterine growth retardation. Until now, abortion is found among both immune and partially immune pregnant women, in holoendemic tropical regions of the world.

22 According to the Authors, low rate of positive retrievals, may be attributed to poor preservation of plasmodial DNA in ancient bone specimens (Sallares, 2004).

Empire. In particular, studies carried out in Italy on a paediatric skeleton, presumably dating between the fifth and seventh century B.C., and those performed on human remnants of a 17 year-old woman dating at the third century B.C., discovered in Pisa, and in a Roman villa located in the town of Settefinestre (Tuscany coast, Italy), showed bone abnormalities typical of thalassemia (Mallegni and Fornaciari 1995; Baggieri and Mallegni, 2001). The same elements have been noticed in 11 human skeletons exhumed in the Pantanello necropolis, close to Greek colony of Metaponto (Southern Italy) (Carter, 1998): all of them were affected by the typical signs of “porotic hyperostosis”, dating between fourth and fifth century B.C., and were probably homozygote for thalassemia²³. This temporal gap in retrieval of same somatic stigmata in human remnants underlines that Greek colonizers²⁴ were the first population inhabiting Italy, to present a significant frequency of thalassemia-related genetic mutations, and consequent bone marrow involvement, as well as a relationship with anemia caused by *Plasmodium falciparum* disease.

To confirm early presence of typical, indirect somatic signs of endemic malaria in Northern Africa and in Middle East, is of great help retrieval of a skeleton of a 20-year-old male with “porotic hyperostosis”, located in the island of Failaka, in the Persian Gulf. These bones, which have been dated by radiocarbon techniques at approximately 2,130±80 years ago, allowed to find fossilized erythrocytes with the typical sickle shape, with aid of electron microscopy studies (Maat and Baig, 1990). We re-

mind that the hemoglobin S is still present with a 2% frequency in some Mediterranean populations, and haplotype analysis demonstrated that sickle cell trait is present in Sicily, Northern Greece, paucisymptomatic Western Arabia, and are reversely linked with the haplotype called “Benin” {detected in Central-Western Africa (Ragusa et al., 1998)}. According to these genetic population studies, the remnants retrieved in Failaka island may demonstrate that these findings could belong to a descendant of a Macedonian soldier coming from Northern Greece, and carrying the characteristic haplotype Benin²⁵ (Sculmann, 2001).

Deficiency of glucose-6-phosphate dehydrogenase (G6PD) enzyme ranks third in importance, among genetic human mutations which are commonly encountered in Mediterranean populations, and confer intrinsic resistance against *Plasmodium falciparum* infection. Italy, which has been selected for presence of two most common G6PD mutations encountered in Mediterranean populations: results of these demanding studies of population genetics (which are not extensively reported), led to the conclusion that G6PD deficiency, together with thalassemia trait and sickle cell anemia trait, were already present in Southern Europe at decline of Roman empire. It probably occurred as an evolutionary response of populations to pressure exerted by malaria endemic (Sallares et al., 2004).

Route of malaria endemic may also be investigated through detection of mutations which were particularly frequent, and are typical of thalassemia among present Mediterranean populations, like

23 Children who were homozygote for thalassemia disease, i.e., those who inherited trait from both parents, suffer from the clinical variant called thalassemia major, or Cooley's anemia. They show a voluminous skull with swelling in the frontal and parietal regions, a large forehead, prominent zygomas, distanced eyes often with an almond-shape aspect, a large and flabby nose, and protruding jaws. This aspect is reproduced on some terracotta heads, like those retrieved in Smyrna archaeological remnants, close to Anatolian coast of Egean sea (Figure 3).

24 Observations carried out on archaeological specimens carefully assessed from an historical medicine point of view, already confirmed that Greeks frequently showed typical somatic tracts of thalassemia. Mirko Grmek and Danielle Gourevitch examined seven small terracotta statues discovered again in Smyrna, on the Anatolian coast of Turkey, dating around Hellenistic period. All these handicrafts preserved “swelling faces, with hypertrophic zygomas, and symmetric prominences on selected (frontal-parietal) head parts, and also had an enlarged, flattened nose basis” (Figure 3). Based on these somatic aspects, two researchers concluded that these small statues presented the pathognomonic traits of homozygote thalassemia (Grmek and Gourevitch, 1998).

25 Alternatively to this last hypothesis, it remains possible that remnants of indigenous human retrieved in Failaka was a thalassemia carrier who, because of molecular features of sickle cell trait (with a different haplotype association), had a genotypic mutation originating from India and Arabia (Maat, 1993). We remind that Indian variant of thalassemia has a phenotype associated with elevated plasma levels of fetal hemoglobin, which inhibits the formation of sickle erythrocytes. On the ground of this second hypothesis, we may explain why the subject retrieved in Persian Gulf area, could have reached adult age.

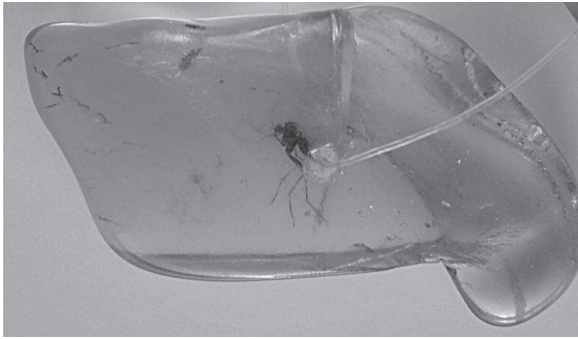


Figure 2 - A mosquito specimen included in an amber fragment.

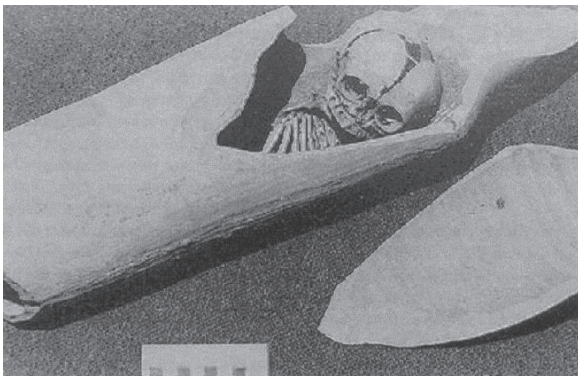


Figure 3 - A model of sepulture in the paleochristian cemetery of Lugnano (Tiber river valley, central Italy). Child's skeleton is contained in an amphora. Image is reproduced from the paper of Sallares (Sallares et al., 2004).



Figure 4 - Child's head bearing typical stigmas of Cooley's anaemia. This archaeological report has been retrieved in Smyrna, aegae coast of Turkey (Paris, Louvre Museum).

Snt110 (G-A) mutation, which is also found in some Italian regions colonized by ancient Greeks. Greater frequency in countries that border the Eastern Mediterranean basin, like Greece, while in Turkey elevated levels of haplotype diversity, as was also demonstrated (Tadmouri et al., 2001).

On the ground of this genetic drift, some authors suggested that this particular mutation had its origin in Greece or Anatolia, and subsequently moved towards Western countries, including Italy, following migration routes originating from Hellenic peninsula starting from the eighth century b.C. (Cao et al., 1989).

On the contrary, CD39 mutation, which represents second most frequent mutation encountered in Mediterranean populations bearing beta-thalassemia trait, is concentrated in Western Mediterranean regions colonized by Phoenicians and achieves its higher level of variety through genetic recombination associated with haplotype (Cao et al., 1989). On the ground of this prevalence we now think that this mutation had its roots in Northern Africa²⁶, and later spread towards major Italian islands and Iberian Peninsula, at the time of Phoenician colonization (Sallares et al., 2004). Therefore, population genetic studies supported scientific evidence of two very relevant subjects. Malaria, its mosquito vectors²⁷, and human genetic mechanisms conferring resistance to malaria infection, reached Southern Europe through two different routes. First one took origin from Tunisia; going through Sardinia and Sicily, it arrived in Southern Italy, and subsequently moved along Italian peninsula along a Southern-Northern route. Second route started from Eastern Mediterranean coast, moved towards West from Anatolia to Greece, and finally reached Italy²⁸ (Sallares et al., 2004).

²⁶ During the glacial era, Northern Africa presumably represented the main shelter of *Anopheles lambrachiae*, and still today it remains the geographic area which allows the preservation of the most affordable habitat for these varieties. We also remind that *Anopheles lambrachiae* is known to be the most efficient vector of malaria in our country (Italy) during recent centuries.

²⁷ The most important malaria vectors in Western and Western Mediterranean basin were represented by two different mosquito species: *Anopheles labranchiae* and *Anopheles sacharovi*. The first species is the most efficient vector in Central and Southern Italy, it originated in Northern Africa, and although remaining active all year round it is reproduced in this geographic area only. The second species, called *Anopheles sacharovi*, comes from near Orient, and was the predominant vector in Greece; it succeeds in surviving the cold winter temperatures of some regions of Southern Europe, being capable of hibernation (Horsfall, 1955).

²⁸ According to the historical literature which could not exploit genetic population studies, older scientist suggested that malaria reached Italy and Greece during the same time period, since it was not possible to imagine that this epidemic infection actually followed different routes and different historical evolutions in two countries which are very close and apparently so similar from a climatic-environmental point of view (Sallares et al., 2004).

The second subject, who supports the title itself, we have selected for our present contribution, leads to understand that *Plasmodium falciparum* infection certainly represented an important agent of natural selection, by acting initially on populations living in Eastern Mediterranean coasts, while this pressure moved towards Italy only in a second time. During this progression of malaria towards Italian peninsula, an important role was played, as mentioned, by two distinct varieties of *Anopheles* mosquitoes which represented major vectors of plasmodia in Southern and Central Italy (*Anopheles sacharovi*), and Northern-Eastern Italy (*Anopheles labranchiae*), respectively. Later appearance of malaria in North-Western Italy, compared to Greece, is related to slow adaptive mechanisms of *Anopheles labranchiae*, which spent some centuries to match climate conditions present in regions where an efficient vector was still lacking, although the majority of conditions which support malaria spread were already present. Given these premises, progressive diffusion of malaria in continental Italy occurred

during late Middle Ages, when endemia became evident in Po river delta, and North-Eastern regions of Italy²⁹.

Close relationship of Ravenna (and later Venice), with the Byzantine empire allowed environmental penetration of *Anopheles sacharovi* mosquito, while *Anopheles labranchiae*, which was predominating vector of Western countries (including Central and Southern Italy), did not succeed in overcoming barrier represented by Tuscany-Emilian Apennines (Sallares et al., 2004).

During late Middle Ages, central Italy was heavily interested by malaria endemia. Dante Alighieri himself, in his Divine Comedy (twenty-ninth chapter of "Inferno") ("Hell" section of poem), clearly reports poor health conditions of people living in Chiana river valley, Maremma, and Sardinia between months of July and September, in poem triplets referred to "Malebolge" ("Malebolge") location³⁰.

According to research studies completed until now, times and modes of malaria spread in Italy appear reasonably known, while it is not the case

29 Already after the flood of 489 a.C., a small group of Benedictine monks settles in the island of Codigoro located in the former Po river delta, to witness their faith through their known motto "ora et labora" (pray and work). Later in the history, during the year 874, this monk community was steadily located at the reknown Pomposa abbey, which has been founded by these Benedictine monastery (as recorded in a letter sent from the Pope John VIII to the Emperor). Thanks to the work of Benedictine monks, the Po valleys were periodically reclaimed from stagnant water (marshes). During the centuries, the advocacy of Benedictine and Cistercian monks was that to impede the degradation of lands in Italy and in many European countries, therefore preventing the massive spread of malaria, although a huge tribute was paid in terms of human life, by monks themselves.

30 "Quando noi fummo sor l'ultima chiostra
di Malebolge, sì che i suoi conversi
potean parere a la veduta nostra,
lamenti saettaron me diversi,
che pietà ferrati avean li strali;
ond'io li orecchi con le man copersi
Qual dolor fora, se de li spedali
di Valdichiana tra 'l luglio e 'l settembre
e di Maremma e di Sardinia i mali
fossero in una fossa tutti 'nsempre,
tal era quivi, e tal puzzo n'usciva
qual suol venir de le marcite membre".

(When we stood above the final cloister
of Malebolge

and all of its lay brothers became discernible to us,
strange arrows of lament, their shafts,
with pity at their tips, pierced me,
so that I pressed my hands against my ears.
If contagion of every hospital
in Valdichiana, from July until September,
and in the Maremma and Sardinia,
were one malarial ditch,
such suffering was in that place.
And from it rose the stench of festering limbs).

for malaria diffusion in Greece and Eastern Mediterranean countries. Based on literature sources, a text of well known “Corpo Ippocratico” (Hippocratic corpus), probably written in fifth century B.C., is the most ancient and reliable reference, which states Greece’s involvement in malaria epidemic³¹. On the other hand, until now we still lack certain bio-molecular evidences which may confirm its presence in older times³².

Indirectly, we can infer that Greece was involved by malaria spread, from study of some archaeological findings retrieved in “Magna Grecia” colonies, where Greek colonists determined their magnificent flourishing³³; as a consequence, we can suppose that Homer’s native country was already involved by worse effects of malaria endemia.

In Central-Southern Italy, malaria-induced pressure was certainly precocious, and a factor which greatly contributed for a favourable environmental context was represented by a temperature increase, occurring concurrently with iron age, and remained until eighth century B.C., and leading to a marine level raising of approximately one meter (Angel, 1966). Such a marine surface rapid elevation supported formation of coastal marshes along Mediterranean basin, in particular when a sandy soil was of concern (i.e. close to river mouths). An interesting occurrence involved Italian Campania region (Southern Italy, close to Naples), and in particular:

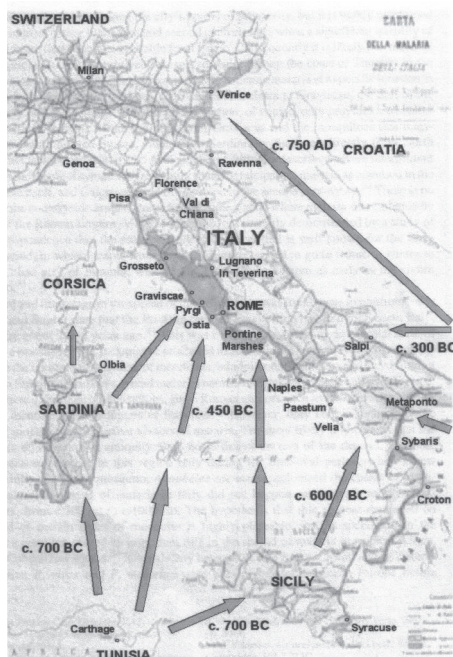


Figure 5 - Geographic chart of Italy, published in 1882 with fittings by Luigi Torelli. Spread routes of malaria are represented, as well as areas originally involved in malaria epidemics.

Sele river valley, where Etruscans built their Southern outpost (located in the town of Pontecagnano, Campania region, Italy). Sele River, together with its tributaries, was subject to frequent overflowing, and these events probably acted in favour of intensification of malaria spread in this territory.

31 Mirko Grmec and Danielle Gourevitch gave their interpretation of paintings which depict Heracles when he kills the mythological Hydra snake with on some Corinthians ceramics, dating from years 630 to 570 B.C. (Figure 5), as an attempt to reclaim malaria-infested marches of Lema country, in Argus region (Grmeck and Gourevitch, 1998).

32 Although molecular biology studies able to date Greece’s involvement in malaria epidemic are not available, observations regarding retrieval of thepical “porotic hyperostosis” remain the most significant reference to re-build the prehistory of malaria in the Hellenic peninsula (Sallares et al., 2004; Angel, 1966;1967).

33 While the relationship between Southern Italy and Greece is certain, one more suggestion regards the possible, common origin of Etruscans and some Anatolic populations (coming from Lydia), coming from Egean sites, based on archaeological studies and recent genetic population investigations. With regard to archaeological documentation, it is mainly represented by the Lemnos stele, located in the so named island of the northern Egean sea, where it was retrieved in the year 1885. This stele reports a text including 198 digits, which give origin from 33 to 40 words, and whose epicoric idiom is strictly related with Etruscan language. Furthermore, excavations carried out in the year 1928 by Italian school of Athens lead to the discovery of vasa fragments of local production which bear similar writings. These archaeological pieces remarkably demonstrate that such a language was actually written and spoken in the Lemnos island, thus leading to strong arguments in favour of Oriental origin of Etruscan people, as concurrently stated by writer Tucidide, and other evidences (Wikipedia, 2009a). Italian genetists analyzed genotypic features of subjects coming from Tuscany, in particular towns of Murlo and Volterra, and Casentino valley, and compared them with those of human samples coming from Sicily, Sardinia, Southern Balkans, and Anatolia. The Results of these investigations demonstrated that specimens coming from Murlo and Volterra are more strictly related to those of populations of near Eastern countries, compared with those coming from other Italian regions. In particular, a genetic variant close to that retrieved in Anatolian residents only has been found in Murlo inhabitants, and from a general point of view specimens from Tuscany show a close affinity with those of the Lemnos island (Pellecchia et al., 2007; Achilli et al., 2007). Data coming from population genetic studies confirm writings of ancient Herodotus, i.e. that Etruscans had their roots in ancient Lydia (Wikipedia, 2009b).

Archaeological excavation of an important Etruscan necropolis, including two distinct groups of buried bodies, first of 275 subjects who spent their life between seventh and sixth centuries b.C., and second accounting (398 dead bodies), dating between fifth and fourth centuries b.C., allowed both paleopathological and paleonutritional investigations, which demonstrated frequent malnutrition syndromes, related to a predominant anaemia picture, where the typical form of “porotic hyperostosis” was proved proportionally frequent (Sabbatani, 2001). These paleopathological evidences were detected in 8.72% of subjects in first mentioned group, and in 3.72% of dead bodies belonging to second group (Fornaciari et al., 1989). Concomitant diffusion of thalassemia in this population was thought to be proportionally frequent, although reclamations and assessment of water and soils realized in these lands by Etruscans³⁴ enabled an improvement of environmental health, and consequently of the “porotic hyperostosis”. Even though these researchers did not allow to establish with absolute certainty the presence of endemic malaria in their geographic area, proportionally elevated frequency of “porotic hyperostosis” found in dead bodies underlines suspicion that a significant percentage of deaths was due to malaria infection, in a high-risk environment.

Some Patterns of Culture Fitting to Endemic Malaria in Human Populations

Although most relevant adaptive measures of populations against malaria infection were mainly of genetic and biological origin; contributions provided by cultural fitting were very relevant, and are extremely interesting to be investigated. This last adaptation involved also an extremely relevant aspect of daily human life, such as dietary habits.

In the light of knowledge coming from nutritional sciences, alimentary choices operated in traditional diet by populations who live in endemic malaria regions, during time were progressively addressed towards food which allows a greater protection³⁵ against plasmodia parasites. Certainly, a dietary regimen enriched with broad beans (*Vicia faba*), typical of countries surrounding Mediterranean sea, confers a remarkable increase of defences against malaria infection (Mikhael, 1986; Katz and Schall, 1979). In fact, some broad beans metabolites, released from gastrointestinal tract, act as potent oxidant agents, which may induce relevant *in vitro* anti-malaric effects, with a further increase of potency when G6PD deficiency is concurrently present (Ginsburg et al., 1996). An experimental case-control study conducted in Thailand demonstrated that dietary intake of broad beans significantly increases anti-malaric defence, especially in subjects who are homozygotes or heterozygotes for the synthesis of hemoglobin E variant (Kitavaporn et al., 1992).

Also vitamin C (ascorbic acid), may act as a pro-oxidizing compound, in presence of an amount of active oxide-reducing iron, so that this last compound a well recognized anti-malaric effect, exerted by acting on advanced plasmodia development stages (Greene, 1999). Although a controlled clinical trial failed in demonstrating a clinically recognized anti-malaric activity of diet supplemented with notable amounts of vitamin C (Levander and Ager, 1993), there is no doubt that Mediterranean diet, usually rich in ascorbic acid-containing food, may be responsible for some protection against malaria, and this cultural basis contributed to reduce disease risk, as extensively reported by specialized literature (Greene, 1999; Golenser, 1997; Har-El and Chevion, 1997).

An elevated number of contributions, whose conclusions are herewith briefly summarized, point

34 Etruscans managed skilled engineering techniques (Agostino and Pontecagnano, 1970). Roman historical writer Titus Livy (liber XXVII, 46, 5) reports on a “Greek ditch” (dugged channels), aimed to convey waters from the lower course of Clanio river, until the open sea.

35 Without entering complicated biochemical mechanisms which act on plasmodia parasite *versus* erythrocyte relationship, three main components played a significant role in adaptation of dietary profile which involved subjects living in endemic areas: i) dietary intake of “oxidative nourishment” profile, which is primary mediator of an anti-malaria diet based on oxidative principles; ii) presence of adequate or elevated dietary levels of transitional metals bearing an active oxidizing-reductive potential (i.e. iron and copper), where iron was particularly useful to catalyze the production of free radicals starting from “oxidative food”, through the so-called Haber-Weiss reaction; iii) selection of a dietary pattern which minimized intake of vitamins and other compounds which play an anti-oxidant activity in parasited red blood cells (Greene, 1999).

out that a low dietary intake of iron represent a sort of adaptive measure of human population against malaria, and other infectious diseases, too. In fact, iron is an essential element enabling plasmodia replication and humans may lower plasma iron levels by increasing its binding with a series of plasma proteins, including lactoferrin, transferrin, and ferritin, which are particularly concentrated in milk. Starting from 1970s, some studies seemed to suggest that subjects suffering from a chronic iron dietary deficiency had a lower incidence of malaria, and also a lower parasitic load, in event of malaria infection. Since milk is a relevant dietary component in populations historically engaged in animal breeding, and milk contains an elevated amount of riboflavin³⁶, also dietary regimens based on milk and its derivatives was thought to represent a preventive measure against malaria parasites (Greene, 1999).

Furthermore, a diet based on an increased intake of fish (in particular, sardines and pilchard, and related species), which is rich in highly pro-oxidative fats, like omega-3 eicosapentanoic acid, and docosaexanoic acid, when associated with intake of food with a proportionally low vitamin K content, has been demonstrated capable to induce a specific protective function against malaria. Mediterranean populations of historical ages could certainly take advantage from an elevated dietary intake of fish, especially in coastal zones. Also frequent resort to salt down and/or kipper available fishes (like cod and herring), increased their availability also during Winter season, when fishing became dangerous and poorly profitable.

Preservation of currently employed food (i.e. that of grain and derivatives, in mud containers), allowed to mount exogenous defences against malaria in a population living in Nubia between the years 350 and 550 b.C. Certainly, the Ballam population was

not aware of such an advantage. In fact, agriculture products like wheat, barley, and millet, which were the basis for production of beer and bread, after their preservation in vases shaped with Nubian mud, were colonized by streptomycetes, leading to a massive growth of these bacteria. Streptomycetes³⁷ are known to produce natural antibiotics like streptomycines (Bassett et al., 1980), which act effectively against Gram-negative and Gram-positive bacteria, Rickettsiae, and Spirochetes, as well as malaria plasmodia, too (Clyde et al., 1971). Although Nubian, Ballam population could not be aware of these notices, consumption of food contaminated with the above-mentioned microorganisms allowed to introduce undoubted selective advantages in prevention of malaria which was a major pediatric morbidity and mortality factor, in this geographical context (Bassett et al., 1980). Observation of nature represented one of the fundamental elements of ancient populations, with particular reference to Egyptians. This last population, thanks to its large cultural and archaeological state, has left extensive documentation, which reached the contemporary world. Even when considering malaria, Egyptians were the first ancient population which has transmitted a written witness of their knowledge. Into the Denderah³⁸ temple, hieroglyphic characters compose the following sentence: "Beware of going outside home after the sunset, during the weeks following Nile river floods". This last sentence certainly represents the oldest anti-malaric preventive precept, and it demonstrates that this evolved people already had developed their nature observation, and already retrieved relevant precepts, aimed at ameliorating their quality of life.

Also literature evidences allow us to appreciate depth of ancient Egyptian knowledge adopted these contrivances: people inhabiting over marsh are

36 Although riboflavin is usually considered as an anti-oxidant, it was actually thought to act as a non-specific pro-oxidant. Some *in vitro* evidences show a riboflavin oxidant activity against malaria, which appeared similar to that exerted by artemisin, when using a special culture medium which includes erythrocytes stratified according to age, and alpha- and beta-thalassemic traits (Greene, 1999).

37 Streptomycetes require a very dry, warm, and alkaline environment to support their growth. They include 60-70% of bacteria retrieved in the 'desert soil' of Nubia. These microorganisms live by metabolizing organic soil particles, and have a positive advantage as opposed to other bacterial species, which have a more rapid growth, but are strictly dependent on a wet and acid environment, and deserve moderate temperatures.

38 Denderah temple's rear portion has been built as early as at end of second century b.C., during Ptolemy's kingdom (twelfth Auletes), from year 80 to 51 b.C.: he was Cleopatra's father (the seventh, last Ptolemaic dynasty's queen). During Roman age some other buildings were added, at time of Augustus, Nero, Domitian, and Trajan emperors.



Figure 6 - Dante and Virgil (from Dante Alighieri, in "Divine Comedy") are mercifully looking to damned souls confined in the tenth Hell's "bolgia", and damned because they were "false manufacturers of every piece of work".



Figure 7 - A representation of a Hydra retrieved in Caere (close to the Central Italian town of Cerveteri), and dating at year 525 b.C. The mythological killing of Lerna's Hydra is represented (preserved at Paul Getty Museum of Malibu (CA, USA).

protected by high towers, where they go and sleep overnight; in fact, because wind mosquitoes cannot fly towards height. Citizens living in the proximity of marshes have found expedients other than towers: everybody has got a net, which is used for fishing during day time; overnight, the same net is placed around the bed (where they sleep under an effective protection). Although we know that mosquitoes may

prick also through a coat or a sheet, these insects cannot even attempt to go through a net.

In conclusion, we can state that adaptive mechanisms of human kind never stopped during time, but evolution of a series of biological factors (plasmodia, vectors), paralleled environmental changes, by searching conditions which were more favourable to their "success". On the other hand, formerly hominids, and later humans, through a series of genetic and immunological adaptations (which have not been extensively treated in this present review), and other cultural and behavioural fittings, guaranteed an adequate evolutionary response, able to successfully overcome pressure imposed by malaria parasite dissemination. However, it remains possible that just at this time a *Plasmodium* species which usually infects primates, possibly through a novel, effective vector, may become able to infect humans, either in a rain forest, or an isolated bare patch, or around a tropical pond located in central Africa, Southern America, or Borneo³⁹. In these hypothetical circumstances, one or more selected mutations, capable to confer this novel plasmodia both human tropism and an increased pathogenicity, may occur. This event may open a new pathway to a novel epidemic, caused by first introduction into a village or a community of a novel *Plasmodium* species, which may act dramatically on health of susceptible mankind (which lacks a specific immune system defence), after being steadily adapted to local environment.

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³⁹ In the year 2004 a first focus of malaria due to *Plasmodium knowlesi* has been recorded in Sarawak state of Indonesia, and in Borneo region of Malaysia, and between November 2004 and March 2005 four deaths were described in Sarawak area, initially attributed to a *Plasmodium malariae* infection. Some doubts raised regarding this attributed ethiology, since *Plasmodium malariae* usually is associated with a low parasitemia and it does not lead to death. Blood samples of these patients were retrospectively examined by a PCR technique, and existence of this novel human *Plasmodium* species was confirmed. Noticeably, *Plasmodium knowlesi* shares a similar microscopic aspect just with *Plasmodium malariae*, and it is therefore at risk to be missed or underestimated in its frequency, and especially in its pathogenicity (Cox-Singh et al., 2008). In a very recent paper published in the outstanding Journal "Nature", genotypic sequence of *Plasmodium knowlesi* has been reported for the first time (Pain et al., 2008). At the same time, N.J. White wrote an "Editorial comment" for Journal of Infectious Disease Society of America, named "Clinical Infectious Diseases", which was entitled: "*Plasmodium knowlesi*: Fifth Human Malaria Parasite" (White, 2008).

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