Malaria infection and human evolution

L’infezione malarica nel contesto dell’evoluzione antropologica

Sergio Sabbatani¹, Roberto Manfredi¹, Sirio Fiorino²

¹Infectious Diseases, S. Orsola-Malpighi Hospital, Bologna, Italy; ²Internal Medicine, Budrio General Hospital, Budrio, Italy

INTRODUCTION

The available paleopathological evidences demonstrate that infectious diseases as a whole became significant as to morbidity and mortality rates for the human kind, only after the cultural progress which led to the development of zootechny, and subsequently to the extension of agricultural techniques [1, 2]¹. The development of agriculture and animal breeding characterized by the domesticking of bovine and cattle and the extensive culture of the most relevant vegetable species, led to a progressive process of sedentary attitude of population². The demographic development which followed the increased food availability allowed the first population aggregations in well circumscribed areas; just in this era, some infectious diseases (predominantly represented by anthropozoonoses mainly transmitted by bovines, ovines, cattle, and dogs domesticated during the Mesolitic age) became epidemic in nature, and produced a significant, negative demographic impact on the resident population. In this framework, the studies conducted by Cohen and Armelagos demonstrated a strong link between infectious diseases and demographic structure of populations [4]. The history of primitive agricultural-based societies, and basically those of the medieval Europe, clearly demonstrate the existing relationship with the cyclic temporal sequence of severe famine episodes, due to nourishment crisis, related to the exaggerated expansion of populations, as opposed to the objective food availability, and with a significant relation with disease epidemics [5]. According to Biraben reports, the cycling occurrence of both famines and epidemics is common of all locations and all ages, since it is supported by some structural frailties of the ancient agricultural production, which were poorly controllable due to their primitive and backward features [6]³. Should all statements reported above be the result of the process of human development dur-

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

²Demographical estimates, carried out between sixties and seventies of the past century, may lead to hypothesize that European population accounted of around eighty million inhabitants, during the mean Neolithic age.

³Luigi Capasso, in order to explain the relationship between famines and epidemic illnesses, has developed a theoretical model, based on the calculation of energy features. The Author underlines that it is indifferent to establish whether the introduction of agriculture was the technological response to a demographic increase, already registered at the end of Paleolithic age, or whether this increase was caused by the earlier animal and vegetable domesticating and culture [5]. According to this model, when the energy amount drawn out of the system (which may be of low entity in the event of an economy based on hunting and harvesting, or medium entity when the system is founded on agriculture and breeding), becomes lower, compared with the amount of energy needed to nourish and maintain alive the entire population. In this last event, a crisis characterize by an extraordinarily elevated mortality rate occurs (it is also the case of epidemics). Based on this theoretical approach, characterized by the binomial demography versus energy taken from the system (i.e. the so-called biomass), we may also fit many historical war conflicts, i.e. the barbarian invasions of the Roman empire, or the origin of the World War II by the nazist Germany, especially when considering the invasion of the Soviet Union occurred in the year 1941, attributable to the richness of that country in agricultural and natural resources (oil- and gas-fields).
In order to underline this last concept we can point of view [3].

Strictly connected to each other, from a tropism greater when they regarded organisms more species evolution is a “coevolution” process [7]. Of course, the reciprocal influences have been considered with K.C. Kim, the comprehensive process; as a consequence, following the considerations of K.C. Kim, the comprehensive species evolution is a “coevolution” process [7]. Of course, the reciprocal influences have been greater when they regarded organisms more strictly connected to each other, from a tropism point of view [3].

In order to underline this last concept we can remind some extensively acquired knowledge. The start of a whatsoever reciprocal influence is matched with a “contact” between two living beings belonging to different species, if from this contact an interaction occurs, or the two living beings are not indifferent; in this last event, three different scenarios may be expected, and all of them are characterized by the start of an historical “coevolutionary” course. In the first case, an organism feeds of the second one (or vice versa), while in the second case both living beings live together without any sort of damage for both organisms; finally, in the third case an organism makes use of some function or resource of another living being, for its own advantage. In this third situation, two different options may result: a damage for the second organism which suffers from the presence of the first one, may occur or not. The first reported case represents the main aspect of an elementary physiological function as the nutrition is; the coevolution mechanism starts when the “predator” organism develops specific mechanisms able to support the efficacy of its aggressive behavior, while in the same time the “prey” mounts a response, by ameliorating its specific defence mechanisms (i.e. natural immunity defence).

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

The third case is representative of the parasitic condition. The 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 the parasited organisms have no 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 pathological evolution.

When coming back to the concept of coevolution, the parasitism represents its ideal model. In fact, in this situation the host-parasite dynamics finds its major expression, and each adaptive change of the 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 the eterochronic variations typical of ontogenesis [3].

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

When the parasites are species-specific, an epidemiological balance becomes evident between host and parasite (evolving toward a parasitism without damage/disease, or a symbiotic relationship)⁴. However, it is also possible that the virulence pathogenicity features of single parasites, i.e. their capacity to induce damage to specific hosts, may be increased by repeated passages of the same organisms in host 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

---

⁴From a theoretical point of view, the older is the first contact with infectious agents, the milder is expected to be the “modern” disease, since in these last conditions a very long time is elapsed, to allow the development of a balance between the host and its potential parasite.
their “old” parasite-host relationship. From this point of view, the Influenza virus model is very relevant and up to date. As early as in the year 1963, Cockburn summarized these concepts as follows: “The infectious diseases affecting modern human kind are the result of all the relationship that our species had in the past and still has, with organisms which acted and still act as parasites” [8]. As a whole, the 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 parasite (including parasites specific for humans), leads to a parallel, true coevolution of both “partners”. Otherwise, the relationship is extended to hosts other than humans; this last issue predominates when the relevant parasite has a spontaneous, extensive host range [3]. These two different modes of relationship were affected by temporal changes, so that the human-parasite dynamics has driven in a significant way the evolution of mankind. On these preliminary basis, we can hypothesize that infectious diseases have represented a very relevant way of selective pressure in the different environmental ecosystems, on a worldwide basis, and may have conditioned the ontogenetic mechanisms too, possibly modifying the evolutionary potential of single species and organisms [3]. Through an accurate revision of the existing literature evidences, we will 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 the long-term evolution of the genus Homo, considering the species Homo habilis, Homo erectus, and Homo sapiens.

THE INFECTIONS BY MALARIA
PLASMODIA IN NATURE

Malaria plasmodia descend from ancient endocellular parasites of the 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 pluricellular organisms, where they were able to survive1. Subsequently, the ancestors of plasmodia acquired the ability to transfer from intestinal cells towards liver cells (through the portal circulation). When colonizing the liver tissue, plasmodia might complete their vital cycles, and may spread into the systemic circulation of their host. The parasite cycle was re-activated when an hematophagous arthropod insect2, by stinging the relevant host, became able to transfer the infection to another individual, which therefore became parasited. On the ground of paleontological evidences, we can suppose that plasmodia, already present since a long time before the appearance of the first vertebrate beings, circulated among primates, transmitted by mosquitoes of the genus Culex. In the New World, this situation remained unmodified for a long time, during the mean and late Tertiary ages, until the genus Anopheles appeared in the Oligocene period (but only in the Old World), and became the vector of plasmodia infection among Primates. This event has been dated at the end of late Tertiary age. Later, during the late Pleistocene age, humans finally appeared in the African continent, and immediately became involved by epidemiological chains of the different plasmodial infections. As a consequence, this assumption explains that the mosquitoes of the genus Culex remained the selected vectors for birds and mammals since the Cenozoic era, while primates and subsequently early hominids were simply involved in pre-existing epidemiological chains [1, 3].

The comparative genotypic analysis of plasmodia, and in particular the comparison of DNA contained in the 18S ribosomal gene, suggests that the divergence of Plasmodium falciparum from its more close plasmodial species, the Plasmodium reichenowi which parasites chimpanzees, occurred approximately five to

---
1As known, the ability to survive for a variable time into phagocyte cells of the immune system has been demonstrated for different bacterial species (i.e. meningoccci, gonoccci, mycobacteria) [8].
2Fossilized mosquitoes have been detected sono in the Eocenic limestone layers of the Green River Formation, in Colorado, USA [9]; in the majority of cases, the insects were represented by the Culex species [10], responsible for the transmission of infection to birds, while, as already known, the transmission of plasmodia among primates is due to the mosquito belonging to the genus Anopheles. Fossilized remnants of this last genus were retrieved in the Old World only. Luigi Capasso supposes that the genus Culex, which appeared during early Tertiary age, guaranteed the spread of plasmodia in this particular time period. Only later, during mean Tertiary age, the circulation of plasmodia will be ensured by mosquitoes of the genus Anopheles [3].
ten million years ago, and in this last case a co-evolution has occurred [11]. As opposed to the evolution of Plasmodium falciparum into the three other species which cause disease in humans (Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale), the passage of these parasite could be happened through a collateral pathway, and possibly directly from other primates to humans (Figure 1).

An intense debate emerged with regard to the most recent molecular evolution of Plasmodium falciparum. A controversial statement points out that the Plasmodium falciparum populations present in the 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 a high degree of genetic diversity, during hundred thousand years of evolution. These apparently divergent statements may be the result of 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 performed, and the Authors concluded that the more 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 be occurred during the last 10,000 years [12].

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 the progression of its more effective vectors in tropical regions of the African continent, such as the expansion of the mosquitoes group belonging to the species called Anopheles gambiae. Since the ecotype living in the forest has been established as the oldest one, we may therefore suggest that an anthropophilic behaviour probably began in a limited forest environment, where the mos-

---

**Figure 1** - A graphic representation of the biological and phylogenetical evolution of Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale (group a parasites), and the more recent pathway performed by Plasmodium falciparum (group b). The first three mentioned plasmodia completed their coevolution in parallel with that of the mankind or, alternatively, human encountered these protozoa during the most ancient phases of evolution (the 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. The Figure is modified from the cited paper by L. Capasso [15].
quitoes has a small number of great-size animals at disposal, and may have a greater tendency to affect humans [13]. The 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 [14]. The results of these multidisciplinary researches lead to conclude that the first contact between *Anopheles gambiae* and *Homo sapiens* occurred in the forest ecotype of tropical Africa, and this event was not necessarily so recent (i.e. dating at the Holocene period), but may be much older, and may have involved earlier human ancestors.

If biomolecular studies of DNA of *Plasmodium falciparum* suggest that this last parasite is much older, the role played by *Anopheles gambiae* remains essential in order to establish how and when the malaria spread had its diffusion, since the increased growth of *Plasmodium falciparum* in the population of the Holocene era was probably supported by the evolution of other ecotypes of *Anopheles gambiae*, different from the original ecotype which had its habitat in the rain forest [11].

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

On the other hand, Luigi Capasso attributes a significant role to the mosquito species *Culex tanzanianae*. This last anthropophagite insect may have been a relevant vector of plasmodia for our ancestors, since it had its endemic area along the East African coast, between the late Pliocene and the early Pleistocene, concurrently with the presence of the first members of the genus *Homo* [5].

An interesting, but still open question, regards which species of mosquito played a key role in the transmission of malaria plasmodia in the

---

1 A recent archaeological research points out that human populations of hunters-harvesters were continuously present in the environment of rain forest, for a long period of time [14].

2 The humans are known to have reached the Americas through the Bering strait around 30,000 years ago; however, adequate findings able to document their presence, are dated at 15,000 years ago.
Plasmodium falciparum and human kind has resolved (from an evolutionistic point of view), after the development of a genetic resistance based on the establishment and the persistence of a polymorphism of genes which encode for the globin protein.

The 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 the most extensive contact with Plasmodium falciparum occurred in a proportionally more recent time, compared with other malaria forms.

It's a general opinion that the massive human malaria infection from animal reservoirs, occurred in Africa after the introduction of agriculture, and the progressive diffusion of a sedentary lifestyle [17]. Probably the massive deforestation carried out in Eastern Africa to acquire cultivable soil, greatly supported the mosquito proliferation. Moreover, the great somatic plasticity of mosquitoes allowed to these haematophagous insects to rapidly adapt to the changing environmental conditions, by occupying the novel ecologic niche ensured by stagnant waters which progressively took space around primitive villages [3, 18].

The subsequent step was the modified habits of some mosquito species, which ceased their nutrition of primate blood, evolving towards the nutrition of human blood. This last occurrence was the crucial point for the disappearance of Plasmodium falciparum as a major parasite of wild primates, to become one of the most important element of natural selection of human kind [3].

In our previous discussion, we examined the possibility that the New World have been interested by Plasmodium falciparum malaria only after the discovery of Columbus occurred in the year 1492, since possible, prior contacts regards 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 may be stated that malaria was a disease unequivocally limited to the Old World, until the year 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 and/or aspecific, but when characteristically recognized, may lead to confirm its role in these skeleton deformities. These bone sequelae 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, sickle cell anemia, constitutional anemia) may also lead to a picture of “porotic hyperostosis”.

Furthermore, also extensive and prolonged intestinal parasite diseases may induce a malabsorption syndrome, which in its more severe forms may support pathological damages not different from those of “porotic hyperostosis” encountered in human malaria [3]. However, even when taking into account these limitations, the paleopathologic investigation on human remnants may address (together with other proofs) to establish the eventual responsibility of Plasmodium falciparum in supporting this pathological condition. Given that the genetic-based hemoglobinopathies represent a reliable marker of population involvement, should Plasmodium falciparum be endemic since long time, among American Indian populations 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 [3]. The
extraction of DNA 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 the last decade of the twentieth century, the molecular biology techniques allowed to reliably establish the existence of an ancient Plasmodium falciparum infection. The laboratory assay named "ParafsightTM-F", which is able to retrieve the antigen of the histidin-rich protein 2 (the so-called protein PfHRP-2) descending from the Plasmodium falciparum trophozoites, may help in the identification of malaria antigen specific 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 "Camarones" 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 [22].

As we may synthetically assess, although biomolecular investigation have reached very advanced scientific standards, the assessment regarding the ancient history of malaria infection is still subject to some uncertainty. In order to reach our objectives, it becomes easier to trace back the ancient figures starting from the present knowledge, linking the scientific evidences coming from molecular biology, with both available historical-medical evidences, and archaeological materials and related studies (including paleopathological 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 the glacial periods of the Quaternary era (around two million years ago) until the 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, the quotations regarding intermittent fevers reported by the ancient literature (Hippocratical reports), dating at fourth and fifth century B.C., some old Indian documents (of difficult dating), and the 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 glaciations era, and the year 500 B.C., although no certain documents exist [11].

While there is a general agreement in retaining the origin of the malignant tertian malaria (due to Plasmodium falciparum) located in tropical Africa [23], the Roman historical Celsus (who lived in the first half of the first century a.C.) [24], made the first differential diagnosis between the clinical course of Plasmodium falciparum-related fever, and the more benign Plasmodium vivax-related disease. The awareness that already during the Roman age a substantial difference between the two main forms of malaria fever had been recognized, comes from the documents of the ancient physician Celsus, as reported around the year 100 a.C. by Archigen of Apamea13. Of certain, the physicians of the ancient Roman world were already aware that patients suffering from a malignant semitertian fever had a worse course in autumn, and this complication was not so frequent in older ages. This consideration suggests that in Italy the pathocoenosis included a novel disease, clearly identified for the first time in this historical period, and the different clinical variants of malaria were endemic in central and southern Italy [11].

Mirko Grmek, one of the most important contemporary experts in the history of Medicine, on the ground of literature evidences, suggests that just malaria fevers interested the Athenian army which besieged the Sicily city of Syracuse, during the Peloponnesian war [26]. On the ground of this observation taken from the literature, and re-enforced by the 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 year 800 and year 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 sixth and fifth century B.C., experienced a rapid decline just because of malaria epidemics [11]. After an earlier historical phase, which involved the centuries preceding 400 B.C. (limited

13Quoted from Aetius of Amida (former Mesopotamian region) [25].
to Sicily and Southern Italy in its coast sites), the malaria diffused in Italy in a South-North direction toward central-western regions, when the so-called second stage of malaria spread had its origin [11]. During the initial time period, until around 400 b.C., the Etruscan cities of Maremma region in Italy, had their period of expansion, as demonstrated by the relevant archaeological findings like vases imported by Greece. Moreover, continued and close contacts with the emerging Roman potency characterized 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 the third and second century. Cato the Senior refers on the presence of malaria fever in Graviscae, the port of the Tuscany coasts during the third and second century. Galen quotes this information in its writings, Cato the Senior refers on the presence of malaria fever in Graviscae, the port of the Etruscan city of Tarquinia, around the year 150 B.C. [27]. 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. We remind that during the preceding centuries the citizens of Syracuse and the Greeks, after defeating the Etruscans in the naval battle of Cuma (474 b.C.), achieved the control of the Thyrenian sea, exerting their check on maritime routes and commercial exchanges with regions where malaria was already an endemic disease, since long time. 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 its writings, three centuries after [28]. Rome was certainly surrounded by territories infested by malaria parasites also during the imperial centuries, and when considering also the general decline following the dissolution of Roman Empire, even more during subsequent centuries. Archive studies of the Vatican correspondence, demonstrated that pilgrims coming from Northern Europe around the eight century a.C., preferred to make their way to Rome during the winter season, in order to avoid “the bad air” which polluted the environment during the summer period of the year [29]. The way followed by malaria parasites towards North-Eastern Italy lasted for a while. Around 2,000 years ago the region of the Po river delta, not far from the city of Ravenna (the last capital of the Western Roman Empire), was reported to be free from malaria. This last information comes from the writers Vitruvio and Strabone, and sounded strange for the time, since the surrounding regions were already marshy [30, 31]. According to Sidonio Apollinare, should mosquitoes be present, they were probably not sufficiently effective in transmitting malaria parasites: in fact, malaria became endemic in these regions only during the Middle Ages, when an anthropophilic mosquito (Anopheles sacharovi), appeared and became permanent along the North-Eastern Italian coasts [32]. In these regions, the malaria epidemic had a very slow spread, and according to Sallares and coworkers, it happened in a range of 1,500 years, from year 500 b.C. and year 1,000 b.C. [11]. Since both Plasmodium vivax and Plasmodium malariae were present in the 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. The speciation of a mosquito belonging to the Anopheles gambiae complex made probably possible the step toward an endemic behaviour of malaria [33]. In fact, only when an efficient vector, capable to survive to cold temperatures of North-Eastern Italy, was selected, Plasmodium falciparum and the two other Plasmodium species had the possibility to take root in geographic areas which were more favourable to their features (often following field abandonment typical of the Middle Ages period14.

DIRECT BIOMOLECULAR EVIDENCES OF THE PRESENCE OF MALARIA IN THE ANCIENT WORLD

In 2008 a study reporting very reliable data of identification of the ancient malaria DNA (aDNA) has been published, together with the evidence of Plasmodium falciparum in Egyptian mummies dated back to 4,000 years ago [34]. The Authors analyzed 91 bone tissue samples of a parallel number of mummies and skeletons retrieved in the different Egyptian sites15.

14In other terms, as Sallares and coworkers wrote, “...all the 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” [11].

15The most ancient site is the pre- and the proto-dynastical site of Abido (an High Egyptian town) (dating around years 3,500-2,800 b. C.), the second site is represented by a grave in West Thebes, of the Mean Kingdom time (years 2,050-1,650 b. C.), while the third site refers to different tomb complexes, also localized at West Thebes, which have been built between the mean and novel Kingdom, and until later periods (from 2,050 up to 500 b. C.).
All specimens have been tested for *Plasmodium* spp. DNA with a polymerase chain reaction (PCR) technique which is based on the use of rDNA of 18S ribosomal subunits, in order to identify unambiguously the eventual presence of an ancient malaria infection. Moreover, in two human remnants coming from two different sites of the New Kingdom, a positivity for malaria infection was found, with specimens matching with osteopathological signs of chronic anaemia [34]. The same assay performed on tissue samples obtained from remnants belonging to earlier time periods, proved negative for malaria infection [34].

On the ground of obtained results, the Authors critically notice that the studies performed during recent years probably overestimated the real amount of malaria infection in the ancient Egyptian world [22]. These studies employed the so-called antigen-2 (a protein rich of histidin), which was detected in over 40% of tested specimens, and in 92% of specimens of human remnants, where signs of the “porotic hyperostosis” were appreciable [22]. Even taking into account this criticism, the same Authors concluded that *Plasmodium falciparum* infection played a significant role in conditioning the limited life expectancy of the ancient Egyptian populations, on the basis of molecular biology examinations conducted with PCR techniques [34].

As mentioned in the above paragraph, if existing evidences document the slow, progressive spread of malaria from Southern to North-Eastern Italy, multiple scientific evidences cumulated during the 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 the Tiber river valley [16], located 70 miles far from Rome. This site includes an extraordinarily large children cemetery of the Roman Italy [17], dating around 450 b.C. [35, 36]. Archeological evidence brought the Authors to conclude that deaths were secondary to an epidemic disease which acted in a proportionally short time period, probably during the Summer [18] (the season burdened by the highest risk of malaria diffusion), as established on the ground of the paleobiologic analysis of remnants of plants and seeds retrieved in the graves [27].

Of 47 human remnants retrieved, 22 were represented by premature children [19], and also the majority of other dead bodies were from neonates. As anticipated, both environmental features and the characteristics of the retrieval, induced the 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 [11]. Molecular biology assays were also performed, through the extraction of DNA genome of *Plasmodium falciparum* from bone specimens. In one case (i.e. burying number 36) the 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 the time of death [39]. The feasibility of DNA amplification of *Plasmodium falciparum* from 1,500-year-old skeleton, suggests a massive malaria infection [20], and not an asymptomatic-paucisymptomatic 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 the Tiber river valley was a place of an epidemic malaria around the year 450 b.C. [11, 39]. The Authors finally observe that the presence of *Plasmodium falciparum* in central Italy, probably associated

---

16The archaeological site is located at 185 meters above the sea in a hill area, 3,5 km far from the river [35]. The river valleys represented the earliest malaric areas in Italy, at the end of Western Roman empire, since the 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 the specific case of Lugnano, this small town is located a few kilometres far from the immission of the Orte river into the Tiber river, so that this particular geographical area was at greater risk for malaria spread.

17In 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 magical rituals, were primarily employed as a spell against tertian fever, as reported by the writer Quinto Sereno [37]. 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.

18Some accurate literature evidences exist regarding the renown unhealthyness of these areas. During the Summer of the year 467 Sidonio Apollinaris run along Italy from Ravenna to Rome, to encounter the emperor Antenio. During his journey, he just reached the malaric regions of ancient Umbria and Tuscany, leaving a striking evidence of the infamous effects of “poisonous miasmas”; severe fever, and insatiable thirst, all clear witnesses of malaria disease.

19Malaria predisposes not only to elevated mortality rates, but also a striking predisposition to absorption among pregnant women, causing an intrauterine growth retardation. Until now, absorption is found among both immune and partially immune pregnant women, in holoendemic tropical regions of the world.

20According to the Authors, the low rate of positive retrievals, may be attributed to the poor preservation of plasmodial DNA in ancient bone specimens [11].
with its main vector, i.e. *Anopheles labranchiae* (still present in the Tiber river valley during the World War II), significantly increased the risk of developing malaria, of the resident population [27]. Since these Italians regions were already populated, epidemic events of malaria were expected at that time.

**INDIRECT GENETIC EVIDENCES OF MALARIA SPREAD IN THE ANCIENT WORLD**

Genetic population studies performed during the past 25 years gave us the elements to understand which was the era of initial selective pressure operated by malaria on Mediterranean populations. The most recent data confirmed the previous suspicions, i.e. this pressure started in proportionally recent times. This observation is acceptable since during previous times the climate was too cold (at least in Europe, and during glacial ages), to allow malaria spread. When underlying that the most ancient osteological evidence of thalassemia, characterized by the typical “porotic hyperostosis”, has been first detected in a site close to the village of Atlit Yam, quite far from the Israel coastal line, and dating around 10,000 years ago, population genetics allowed to find a lack of aplotype diversity which is usually associated with the largest number of mutations conferring resistance to malaria in the modern Mediterranean populations [40]. These evidence suggest that these changes probably represented an evolution started from a time not beyond 4,200 years ago [41]. All major determinants of inherited genetic resistance were already present in the Mediterranean basin at the time of Roman empire. In particular, studies carried out in Italy on a paediatric skeleton, presumably dating between 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), showed bone abnormalities typical of thalassemia [42, 43]. The same elements have been noticed in 11 human skeletons exhumed in the Pantanello necropolis, close to the Greek colony of Metaponto [44]: all of them were affected by the typical signs of “porotic hyperostosis”, dating between the fourth and the fifth century b.C., and were probably homozygotes for thalassemia. This temporal gap in the retrieval of the same somatic stigmata in human remnants underlines that the Greek colonizers were the first population inhabiting Italy, to present a significant frequency of thalassemia-related genetic mutations, and the consequent bone marrow involvement, as well as a relationship with anaemia caused by *Plasmodium falciparum* disease.

To confirm the early presence of the typical, indirect somatic signs of endemic malaria in Northern Africa and in the Middle East, is of great help the retrieval of a skeleton of a 20-year-old male with “porotic hyperostosis”, located in the island of Failaka, in the Persian Gulf. The 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 the aid of electron microscopy studies [46]. We remind that the haemoglobin S is still present with a 2% frequency in some Mediterranean populations, and the aplotype analysis demonstrated that the sickle cell trait is present in Sicily, Northern Greece, and Western Arabia, and are reversely linked with the aplotype called “Benin” (detected in Central-Western Africa [47]. According to these genetic population studies, the remnants retrieved in the Failaka island may demonstrate that these findings could belong to a descendant of a Macedonian soldier coming from Northern Greece, and carrying the characteristic aplotype Benin [38].
The deficit of glucose-6-phosphate dehydrogenase (G6PD) enzyme ranks third in importance, among genetic human mutations which are commonly encountered in the Mediterranean populations, and confer intrinsic resistance against *Plasmodium falciparum* infection. The above-mentioned children of Lugnano have been selected for the presence of the two most common G6DP mutations encountered in Mediterranean populations. The results of these demanding studies of population genetic (which are not extensively reported), led to the conclusion that G6PD deficiency, together with the thalassemia trait and the sickle cell anemia trait, were already present in Southern Europe at the decline of Roman empire. It probably occurred as an evolutionistic response of populations to the pressure exerted by the malaria endemic [11].

The route of malaria endemic may be also investigated through the detection of mutations which were particularly frequent, and are typical of thalassemia among present Mediterranean populations, like the B+IVSnt110 (G-A) mutation, which is also found in some in Italian regions colonized by ancient Greeks. This mutations has its major and greater frequency in the countries which border the Eastern Mediterranean basin like Greece, while in Turkey an elevated levels of aplotype diversity was also demonstrated [41].

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 the migration routes originating from the Hellenic peninsula starting from the eighth century B.C. [49].

On the contrary, the CD39 mutation, which represents the second most frequent mutation encountered in Mediterranean populations bearing the beta-thalassemia trait, is concentrated in the Western Mediterranean regions colonized by Phoenicians and achieves its higher level of variety through genetic recombinations associated with the aplotype [49]. On the ground of these prevalence, we now think that this mutation had its roots in Northern Africa [24], and later spread towards the major Italian islands and the Iberian peninsula, at the time of Phoenician colonization [11].

Therefore, population genetic studies supported the scientific evidence of two very relevant subjects. Malaria, its mosquito vectors [25], and...
human genetic mechanisms conferring resistance to malaria infection, reached Southern Europe through two different routes. The first one took origin from Tunisia, and going through Sardinia and Sicily arrived in Southern Italy, and subsequently moved along the Italian peninsula along a Southern-Northern route. The second route started from Eastern Mediterranean coast, moved towards West from Anatolia to Greece, and finally reached Italy26 [11]. The second subject, which 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 the 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 the two distinct varieties of Anopheles mosquitoes which represented the major vectors of plasmodia in Southern and Central Italy (Anopheles sacharovi), and Northern-Eastern Italy (Anopheles labranchiae), respectively. The later appearance of malaria in North-Western Italy compared to Greece, is related to the 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 the spread of were already present. Given these premises, the progressive diffusion of malaria in continental Italy occurred during the late Middle Ages, when the endemic became evident in the Po river delta, and the North-Eastern regions of Italy27.

The close relationship of Ravenna (and later Venice), with the Byzantine empire allowed the environmental penetration of Anopheles sacharovi mosquito, while Anopheles labranchiae, which was the predominating vector of Western countries (including Central and Southern Italy), did not succeed in overcoming the barrier represented by the Tuscany-Emilian Apennines [11]. During the late Middle Ages, central Italy was heavily interested by the malaria endemic. Dante Alighieri himself, in his Divine Comedy (twenty-ninth chapter of the “Inferno” (“Hell” section of the poem), clearly reports the health poor healthy conditions of people living in the Chiana river valley, Maremma, and Sardinia between the months of July and September, in the poem triplets referred to the “Malebolge” (“Malebolge”) location28. The Figure 5 represents Dante and Virgil on a high steep place, while they look and pity the damned souls of false manufacturers, who were damned to spend their eternal punishment in this Hell round of the Divine Comedy. According to research studies completed until now, the times and modes of malaria spread in Italy appear reasonably known, while it is not the case for the malaria diffusion in Greece and Eastern Mediterranean countries. Based on literature sources, the text of the well known “Corpo Ippocratico” (Hippocratic corpus) of the fifth century B.C. are the most ancient and reliable reference, which state the involvement of Greece in the malaria epidemic29. On the other hand, until now we still lack of certain biomolecular evidences which may confirm its presence in older times30.

26 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 [11].

27 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, these monk community was steadily located at the renown 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.

28 “Quando noi fummo sor l’ultima chiostra / di Malebolge, si 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 lì spedali / di Valdichiana tra ’l luglio e ’l settembre / e di Maremma e di Sar-”

29 On the other hand, until now we still lack of certain biomolecular evidences which may confirm its presence in older times.

30 Although molecular biology studies able to date the involvement of Greece in the malaria epidemic are not available, the observations regarding the retrieval of the typical “porotic hyperostosis” remain the most significant reference to re-build the prehistory of malaria in the Hel-
Indirectly, we can infer that Greece was involved by malaria spread, from the study of some archaeological findings retrieved in the "Magna Grecia" colonies, where the early arrival of Greek colonists determined their magnificent flourishing; as a consequence, we can supposed that the Homer’s native country was already involved by the worse effects of malaria endemic.

In Central-Southern Italy, the malaria-induced pressure was certainly precocious, and a factor which greatly contributed to a favourable environmental context was represented by a temperature increase, occurring concurrently with the iron age and remained until the eight century B.C., and leading to a raising of the marine level of approximately one meter [52]. Such a rapid elevation of marine surface supported the formation of coastal marshes along the Mediterranean basin, in particular when a sandy soil was of concern (i.e. close to the river mouths). An interest occurrence involved the Italian Campania region, and in particular the Sele river valley, where Etruscans built their Southern outpost (located in the town of Pontecagnano). The Sele river, together with its tributaries, was subject to frequent overflowing, and these events probably acted in favour or the intensification of malaria spread in this territory. The archaeological excavation of an important Etruscan necropolis, including two distinct groups of buried bodies, the first of 275 subjects who spent their life between the seventh and the sixth century B.C., and the second accounting of 398 dead bodies, dating between the fifth and the fourth century 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 [57]. These paleopathological evidences were detected in 8.72% of subjects in the first mentioned group, and in 3.72% of dead bodies belonging to the second group [58]. The concomitant diffusion of thalassemia in this population was thought to be proportionally frequent, although the reclamations and the assessment of water and soils realized in these lands by Etruscans enabled an amelioration of

---

While the relationship between Southern Italy and Greece are certain, one more suggestion regards the possible, common origin of Etruscans and some Anatolic populations (coming from Lydia), coming from Egean sites, based on archeological studies and recent genetic population investigations. With regard to archeological 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 to 33 to 40 words, and whose epicoric idiom is strictly related with the Etruscan language. Furthermore, excavations carried out in the year 1928 by the Italian school of Athens lead to the discovery of vasa fragments of local production which bear similar writings. These archeological pieces remarkably demonstrate that such a language was actually written and spoken in the Lemnos island, thus leading to strong arguments in favor of the Oriental origin of the Etruscan people, as concurrently stated by the writer Tucidide, and other evidences [53]. Italian genetists analyzed the genotypic features of subjects coming from Tuscany, in particular the towns of Murlo and VOLTERRA, and the 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 the specimens coming from Murlo and VOLTERRA are more strictly related to those of the 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 the specimens from Tuscany show a close affinity with those of the Lemnos island [54, 55]. Data coming from population genetic studies confirm the writings of the ancient Herodotus, i.e. that Etruscans had their roots in the ancient Lydia [56].

The Etruscans managed skilled engineering techniques [59]. The Roman historical writer Titus Livy (liber XXVII, 46, 5) reports on a “Greek ditch” (dug channels), aimed to conmney waters from the lower course of Clanio river, until the open sea.
environmental healthy, and consequently of the “porotic hyperostosis”. Even though these researchers did not allow to establish with absolute certainty the presence of an endemic malaria in this geographic area, the proportionally elevated frequency of the “porotic hyperostosis” found in dead bodies underlines the 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 the main adaptive measures of populations against malaria infection were mainly of genetic and biological origin, however the contributions provide by cultural fitting was very relevant and interesting to be investigated. This last adaptation involved also an extremely relevant aspect of daily human life, such as the dietary habits.

In the light of knowledge coming from nutritional sciences, the alimentary choices operated in the traditional diet by populations who live in endemic malaria regions, during time were progressively addressed towards food which allow a greater protection against plasmodia parasites. Certainly, a dietary regimen enriched by broad bens (*Vicia faba*), typical of countries surrounding the Mediterranean sea, confers a remarkable increase of defences against malaria infection [61, 62]. In fact, some broad bens metabolites, released from the gastrointestinal tract, act as potent oxidant agents, which may induce relevant *in vitro* antimalaric effects, with a further increase of potency when G6PD deficiency is concurrently present [63]. An experimental case-control study conducted in Thailand demonstrated that the dietary intake of broad bens significantly increases antimalaric defence, especially in subjects who are homozygotes or heterozygotes for the synthesis of the haemoglobin E variant [64].

Also vitamin C (ascorbic acid), may act as a prooxidizing compound, in presence of an amount of active oxide-reducing iron, so that this last compound has a well recognized anti-malaric effect, exerted by acting on the advanced development stages of plasmodia [60]. Although a controlled clinical trial failed in demonstrating a clinically recognized antimalaric activity of diet supplemented with notable amounts of vitamin C [65], there is no doubt that Mediterranean diet usually rich of ascorbic acid-containing food, may be responsible for some protection against malaria, and this cultural basis...

---

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

**Figure 7** - A representation of an Hydria retrieved in Caere (close to the Central Italian town of Cerveteri), and dating at year 525 B.C. The mythological killing of Lerna’s Hydria is represented (preserved at Paul Getty Museum of Malibu (CA, USA)).
contributed to reduce the disease risk, as extensively reported by the literature [60, 66, 67]. An elevated number of contribution, whose conclusions are herewith briefly summarized, point 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, the iron is an essential element enabling the replication of plasmodia, and humans may lower plasma iron levels by increasing its binding with a series of plasma proteins, including lattoferrin, transferrin, and ferritine, which are particularly concentrated in the milk. Starting from the 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 the 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 riboflavin\textsuperscript{a}, also dietary regimens based on milk and its derivatives was thought to represent a preventive measure against malaria parasites [60].

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 the omega-3 eicosapentanoid acid, and the docosaexanoic acid, when associated with the intake of food with a proportionally low vitamin K content, has been demonstrated capable to induce a specific protective function against malaria. The Mediterranean populations of historical ages could certainly take advantage from an elevated dietary intake of fish, especially in coastal zones. Also the frequent resort to salt down and/or kipper the available fishes (like cod and herring), increased their availability also during the Winter season, when fishing became dangerous and poorly profitable.

The 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 the production of bear and bread, after their preservation in vases shaped with Nubian mud, were colonized by streptomycetes, leading to a massive growth of these bacteria. Streptomycetes\textsuperscript{b} are known to produce natural antibiotics like streptomycines [68], which act effectively against Gram-negative and Gram-positive bacteria, Rickettsiae, and Spirochetae, as well as malaria plasmodia, too [69]. Although the Nubian, Ballam population could not be aware of these notices, the consumption of food contaminated with the above-mentioned microorganisms allowed to introduce undoubtful selective advantages in the prevention of malaria which was a major paediatric morbidity and mortality factor, in this geographical context [68]. The observation of nature represented one of the fundamental elements of ancient populations, with particular reference to Egyptians. This last population, thanks to their large cultural and archeological state, have 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 Denderah\textsuperscript{c} temple, hieroglyphic characters compose the following sentence: “Beware of going outside home after the sunset, during the weeks following Nile rive 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 to ameliorate their quality of life.

Also literature evidences allow us to appreciate the depth of ancient Egyptian knowledge. The Greek writer Erodoto (II, 95) reports that Egyptians, who had an intensive fishing practice, were able to defend themselves from mosquitoes. He reports: “Against mosquitoes, which are extremely numerous, they adopted these contrivances: people inhabiting over a marsh are

\textsuperscript{a}Although riboflavin is usually considered as an antioxidants, it was actually though 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 [60].

\textsuperscript{b}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 metabolising the organic particles of the soil, 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.

\textsuperscript{c}The rear portion of the Denderah temple has been built as early as at the end of the second century b.C., during the kingdom of Ptolemy the twelfth Auletes, from year 80 to 51 b.C.: he was the father of Cleopatra the seventh, the last queen of the Ptolemaic dynasty. During the Roman age some other buildings were added, at the time of Augustus, Nero, Domitian, and Trojan emperors.
protected by high towers, where they go and sleep overnight; in fact, because of wind mosquitoes cannot fly towards to height. Citizens living in the proximity of marshes have found expediens other than towers: everybody has got a net, which is used for fishing during the 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 insect cannot even attempt to go through a net".

In conclusion, we can state that the adaptive mechanisms of the human kind never stopped during the time, but the evolution of a series of biological factors (plasmadia, vectors), paralleled the 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 the present review), and other cultural and behavioural fittings, guaranteed an adequate evolutionary response, able to successfully overcome the pressure imposed by the 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 Borneo. In these hypothetical circumstances, one or more selected mutations, capable to confer to this novel plasmodia both human tropism and an increased pathogenicity, may occur. These event may open a new pathway to a novel epidemic, caused by the first introduction into a village or a community of a novel *Plasmodium* species, which may act dramatically on the health of the susceptible mankind (which lacks of a specific immune system defence), after being steadily adapted to the local environment.

**Key words:** malaria, plasmodia parasites, anthropology, evolution, human kind.

**Acknowledgements**

The Authors wish to thank Dr. Simonetta Righi (Internal Medicine Section, Centralized Medical Library, University of Bologna, Bologna, Italy), for her expert advice and valuable co-operation.

---

During the evolution of the genus *Homo*, with regard to the species *habilis*, *erectus* and *sapiens*, malaria has played a key biological role in influencing human development. The plasmodia causing malaria have evolved in two ways, in biological and phylogenetic terms: *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* appear to have either co-evolved with human mankind, or encountered human species during the most ancient phases of *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 the Mesolithic and the beginning of the Neolithic age. The authors show both direct and indirect biomolecular evidence of malarial infection, detected in buried subjects, dating to ancient times and brought to light in the course of archaeological excavations in major Mediterranean sites.

In this review of the literature the authors present scientific evidence confirming the role of malaria in affecting the evolution of populations in Mediterranean countries. The people living in several different Mediterranean regions, the cradle of western civilization, have been progressively influenced by malaria in the course of the spread of this endemic disease in recent millennia. In addition, populations affected by endemic malaria progressively developed cultural, dietary and behavioural adaptation mechanisms, which contributed to diminish the risk of disease. These habits were probably not 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 greater resistance to malaria. All these factors have diminished the unfavourable demographic impact of the disease, also positively influencing the general development and growth of civilization.

---

**SUMMARY**

In the year 2004 a first focus of malaria due to *Plasmodium knowlesi* has been recorded in the Sarawak state of Indonesia, and in the Borneo region of Malaysia, and between November 2004 and March 2005 four death were described in the Sarawak area, initially attributed to a *Plasmodium malariae* infection. Some doubts raised regarding this attributed aetiology, since *Plasmodium malariae* usually is associated with a low parasitemia and it does not lead to death. The blood samples of these patients were retrospectively examined by a PCR technique, and the 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 [70]. In a very recent paper published in the outstanding journal "Nature", the genotypic sequence of *Plasmodium knowlesi* has been reported for the first time [71]. At the same time, N.J. White wrote an "Editorial comment" for the Journal of the Infectious Disease Society of America, named "Clinical Infectious Diseases", which was entitled: "Plasmodium knowlesi: The Fifth Human Malaria Parasite" [72].
Lungo il corso dell’evoluzione del genere Homo (specie habilis, erectus e sapiens), la malaria ha giocato un ruolo biologico chiave sullo sviluppo antropologico. I Plasmodi malarici a loro volta si distinguono per due percorsi evolutivi, da un punto di vista biologico e filogenetico. In particolare, Plasmodium vivax, Plasmodium malariae, e Plasmodium ovale, si sarebbero caratterizzati per una co-evoluzione con il genere umano, o avrebbero parasitato la specie umana nelle fasi più antiche dell’evoluzione del genere Homo. D’altra parte, Plasmodium falciparum è stato trasmesso all’uomo dai primati in periodi storici più recenti, probabilmente tra la fine dell’era Mesolitica e l’inizio dell’era Neolitica. Gli Autori riportano evidenze biomolecolari dirette ed indirette dell’esistenza dell’infezione malarica, rilevate in sepolture del Mondo Antico, portate alla luce nel corso di scavi archeologici effettuati in importanti siti del bacino del Mediterraneo. In questa rassegna della letteratura, vengono organizzate le evidenze di letteratura attualmente disponibili: esse confermano il ruolo svolto dalla malaria nell’influenzare l’evoluzione delle popolazioni residenti nel bacino del Mediterraneo. Popolazioni che vivevano in diverse regioni del bacino del Mar Mediterraneo, che rappresentavano il fulcro della civiltà occidentale, hanno subito una progressiva influenza da parte dell’infezione malarica, nel corso della diffusione di questa malattia endemica nei passati millenni. Oltre a ciò, popolazioni interessate da forme endemiche di malaria, hanno progressivamente sviluppato meccanismi dettati dall’adattamento di ordine culturale, alimentare, e comportamentale, che contribuivano a ridurre il rischio di contrarre il rischio di tale parassitosi. Questi mutamenti di abitudine non erano probabilmente attuati in piena consapevolezza. Ciò nonostante, si può pensare che sia i cambiamenti comportamentali sia le modificazioni biologiche causate dalla presenza di plasmodi malarici, abbiano favorito l’emergere di gruppi di popolazione dotati di più elevati livelli di resistenza nei confronti della malaria stessa. Tutto questo insieme di fattori ha infine contenuto l’impatto demografico sfavorevole della malattia, influenzando nel contempo in misura positiva il progresso e lo sviluppo generale della civiltà.

**REFERENCES**