BURGER'S MEDICINAL CHEMISTRY AND DRUG DISCOVERY

Fifth Edition

Volume 5: Therapeutic Agents

Edited by

Manfred E. Wolff

Teshnipharm Consultants
Laguna Beach, California

A WILEY-INTERSCIENCE PUBLICATION

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Preface

Volume 5 of this series is concerned primarily with CNS drugs and endocrine agents that were not considered in Volume 3. As was indicated there, the advent in the 1950s of orally active agents for treatment of mental illness and intervention into endocrine processes brought about profound changes in everyday life in Western-type societies. Today, in an era of medical cost containment, these changes continue and are increasingly important.

The treatment of all aspects of mental illness, from everyday anxiety to the most deepseated psychoses, is now intimately associated with effective drug therapy. Large economic advantages provided to society by outpatient treatment and shortened psychiatric intervention involving the new agents whose discovery and development is chronicled in these volumes have resulted in a paradigm shift in societal attitudes and legislation regarding mental illness. Important advances in neurochemistry continue to be made, and will promptly be incorporated into the CNS drug discovery process.

In the endocrine area also, basic discoveries regarding the genetic basis of diseases like rheumatoid arthritis will revolutionize our view of how such chronic inflammatory diseases should be treated, and provide entirely new avenues for antisense and gene therapy approaches.

All of this is to say that one understands increasingly the concept of "future shock" enunciated by Tofler a quarter of a century ago—the idea that things are changing at an ever increasing rate. Certainly that is the case in medicinal chemistry and drug discovery. Not only is the pace of change growing, but the commitments of thought leader scientists in key areas have enlarged correspondingly. What this means is that the preparation of an up-to-date, broad-based treatise like *Burger's* becomes more and more difficult because of rapid advances and busy authors, and to a certain extent it causes one to despair that the field can ever be captured comprehensively in printed form. In the 1950s Louis Fieser could do that for steroids with a small book entitled *Steroids*, and Alfred Burger could do the same for medicinal chemistry in a volume by that name. Today the editor, a whole battalion of authors from around the globe, and a dedicated publisher's staff struggle to present the field in a multivolume treatise.

But these difficulties notwithstanding, we hope that for the most part we have accomplished our goal of presenting the salient information for the most important areas of drug discovery and medicinal chemistry. Although we are fully aware that new data are constantly being generated, we hope that these volumes will serve as a convenient, comprehensive, hard copy beginning point that can be readily supplemented by the latest knowledge from online sources.

Again I thank my friends, the dedicated authors, who generously took time from their already overcrowded schedules to pass their expert knowledge on to others. I am grateful to Michalina Bickford, Managing Editor with John Wiley & Sons, for her work in connection with this series. As always I thank my wife, Gloria, for her steadfast support and encouragement in everything I do.

MANFRED E. WOLFF

Laguna Beach, California

Burger's Medicinal Chemistry and Drug Discovery

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CHEMOTHERAPEUTIC AGENTS, Pt. 3

CHAPTER FIFTY-NINE

Antimalarial Agents

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If we take as our standard of importance the greatest harm to the greatest number, then there is no question that malaria is the most important of all infectious diseases." (1)

Ah, poor heart! he is so shaked of a burning quotidian tertian, that it is most lamentable to behold." (2)

1 INTRODUCTION

Malaria is one of the most serious, complex, and refractory health problems facing humanity this century. Some 300–500 million of the world's people are infected by the disease, presenting over 120 million clinical cases annually. It is estimated that between 1.5 and 2.7 million people die from malaria every year, either directly or in association with acute respiratory infections and anemia, and up to 1 million of those deaths are among children younger than five years old. More persons die from malaria each year than have died from AIDS in the last seventeen years. Malaria is a leading cause of morbidity and mortality in the developing world, particularly in tropical Africa, and it remains the single outstanding tropical disease control priority. General information about malaria can be found on the World Wide Web at sites maintained by the United States Centers for Disease Control (3) and by the World Health Organization (4). Reference (5) is also a valuable resource.

1.1 The Disease

Human malaria is caused by four species of protozoan parasites of the Plasmodium genus. These are *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae,* each of which presents slightly different clinical symptoms. *P. falciparum* is the most widespread of the four geographically and the most pernicious, causing the majority of malaria-related morbidity and mortality. Other Plasmodia species specifically infect a variety of birds, reptiles, amphibians, and mammals.

Parasites are transmitted from one person to another by an insect vector, the female anopheline mosquito. In most malarious areas, several species are able to transmit the parasite and the exact species responsible vary from region to region. Male mosquitoes do not transmit the disease. These mosquitoes are present in almost all countries in the tropics and subtropics and they bite during nighttime hours, from dusk to dawn. It has been demonstrated that transmission can occur from transfusion of infected blood or from mother to child *in utero,* although these instances are very rare when compared with mosquito inoculation. The parasites develop in the gut of the mosquito and are passed on in the saliva when an infected mosquito bites a person. Uninfected mosquitoes become infected by taking a blood meal from an infected human. The parasites are carried by the blood to the victim's liver where they invade the cells and multiply. After 9–16

days they return to the blood and penetrate the red cells, where they again multiply and begin destroying the red cells.

The signs and symptoms of malaria illness are variable, but most patients experience fever. Other symptoms often include headache, back pain, chills, muscle ache, increased sweating, malaise, nausea, and sometimes vomiting, diarrhea, and cough. Early stages of malaria may resemble the onset of the flu. Between paroxysms, the patient may remain febrile or may become asymptomatic. Early in an infection, the cyclic patterns of fever may not be noticeable but later, a clear cyclic trend with symptoms recurring at regular intervals occurs. Of the four species of parasite, only falciparum malaria can progress rapidly to the cerebral stage, where infected red cells obstruct the blood vessels in the brain. Untreated cases can progress to coma, renal failure, liver failure, pulmonary edema, convulsions, and death. Although infections with *P. vivax* and *P. ovale* often cause less serious illness, parasites may remain dormant in the liver for many months, causing a reappearance of symptoms months or even years later. Cases of severe disease including cerebral malaria require hospital care.

Malaria is diagnosed by the clinical symptoms and by microscopic examination of the blood. Stained thick and thin blood smears are used to diagnose malaria and to quantify the level of parasitemia. Giemsa-stained thin smears are used to differentiate between the species of parasite. Clinical symptoms are an inaccurate means of diagnosis by themselves, although in the absence of adequate laboratory facilities, as is the case in many malarious regions, it is the only means available. Malaria can normally be cured by antimalarial drugs. The symptoms quickly disappear once the parasites are killed. The standard measures of clinical antimalarial drug efficiency are fever clearance time and parasite clearance time. In certain geographic regions, however, the parasites have developed resistance to antimalarial agents, particularly chloroquine. Patients in these areas require treatment with newer, often more expensive drugs.

In the last several years, it has been noted that microscopic examination of blood is an inadequate method for detecting low levels of parasitemia. The lack of sensitivity seldom affects treatment and diagnosis in acute cases but it does limit understanding of the degree to which malaria is chronic. In an endemic area, polymerase chain reaction studies revealed that more than 90% of the exposed population at any one time was chronically infected with *P. falciparum* (6).

In treating malaria, curing patients is often difficult to define. The relief of symptoms of a malaria attack is a "clinical cure." Parasites may remain, even after symptoms have resolved, either in blood cells or in liver tissue, and recrudescences and/or relapses results in the re-establishment of the infection. A "radical cure" is when the parasites are completely eliminated from the body so that relapses cannot occur. Obviously, a radical cure is the ideal therapeutic endpoint.

The choice of antimalarial agent(s) for treatment in each particular case is determined by a multiplicity of factors such as the parasite species causing the infection, the acquired immune status of the patient, the susceptibility of the parasite strain to antimalarial agents, the facilities and resources available for health care, and the genetic make-up of the patient, to list a few. During pregnancy, women are at high risk of death from falciparum malaria. Also at risk are children who are prone to severe attacks until they develop immunity. Nonimmune travelers to malarious areas are similarly vulnerable.

1.2 The Parasite

The four species of human malaria parasites are evolutionarily, morphologically and clinically distinct. *P. vivax, P. malariae,* and *P. ovale* are closely related on an evolutionary

basis to a number of simian malarias. When comparing small subunit ribosomal RNA gene sequences, *P. vivax* is a closer to *P. fragile,* a parasite of toque monkeys, than to either *P. ovale* or *P. malariae.* It has been suggested that *P. malariae* was derived from a West African chimpanzee malaria. And a plasmodium of New World monkeys, *P. brasilianum,* may in fact be *P. malariae* that has adapted to a new host over the last few hundred years. These parasites most likely arose alongside the primate hosts an estimated 30 million years ago. *P. falciparum* appears more closely related to avian malarias and is of more recent origin, perhaps within the last 10,000 years (7).

Recently, a new species, dubbed *P. vivax*-like parasite, has been described that infects humans (8). Its morphological characteristics show it to be similar to *P. vivax,* but analysis of its DNA indicates that the sequence for the circumsporozoite protein (CS) gene is quite different from that of *P. vivax.* Rather, its CS gene appears to be identical to that of a parasite isolated from toque monkeys, *P. simiovale.* The CS protein is the major surface protein of the sporozoite stage of the parasite and has been studied as a source of malaria vaccine antigens.

The life cycle of the parasite in both mosquitoes and humans is complex (Fig. 59.1) as is the terminology of the various parasite development stages (9). When an infected mosquito bites, sporozoites are injected into the blood stream of the human victim and then travel to liver tissue where they invade parenchymal cells. During development and multiplication in the liver, known as the preerythrocytic stage, the host is asymptomatic. After a variable period of time, 6–8 days for vivax, 9 days ovale, 12–16 days malariae, 5–7 days falciparum, merozoites (5,000 to 40,000 per sporozoite) are released from the liver and the parasites take up residence in the red blood cells (erythrocytic stage). In nonrelapsing malarias (falciparum and malariae), no parasites are left in the liver; the infection moves entirely into the blood stage. In relapsing malarias, some of the merozoites (or sporozoites) differentiate into a dormant nondividing stage (hypnozoites), providing a reservoir of parasites in the liver that can be activated for up to five years following the initial infection. Invasion of the red cell by a merozoite results in the development of the trophozoite stage. The parasite feeds upon the protein portion of hemoglobin and hemozoin, a waste product, accumulates in the host cell cytoplasm. After the parasite undergoes nuclear divisions, the red blood cell bursts and merozoites, parasite waste, and cell debris are released. The presence of the debris is the cause of the episodes of fever and chills associated with malaria. The merozoites released by the red cell rupture go on to infect more erythrocytes. Time intervals between cell rupture (fever), infection of other erythrocytes, and then their rupture (new bouts of fever) are characteristic of the parasite species. A few merozoites become differentiated into male and female gametocytes, forms that are dormant in humans. When a mosquito takes a blood meal from the infected human, the gametocytes begin sexual reproduction in the digestive track of the mosquito. Ultimately, sporozoites form and then reside in the mosquito saliva, ready for a new round of infection.

In discussing malaria, the terms recrudescence and relapse are used to describe the return of disease symptoms from different reservoirs of residual parasites. When a patient has been symptom-free for a period of time greater than the usual periodicity of the paroxysms and then clinical symptoms of malaria return, the situation is termed "recrudescence" if the reestablished infection is a result of surviving erythrocytic forms of the parasite. If symptoms reappear because of the continuing presence of parasites in liver tissue, the term "relapse" is used. Because only *P. vivax* and *P. ovale* have hypnozoites that reside in the liver, these are the true relapsing malarias.

Plasmodium vivax, benign tertian malaria, or simply tertian malaria presents

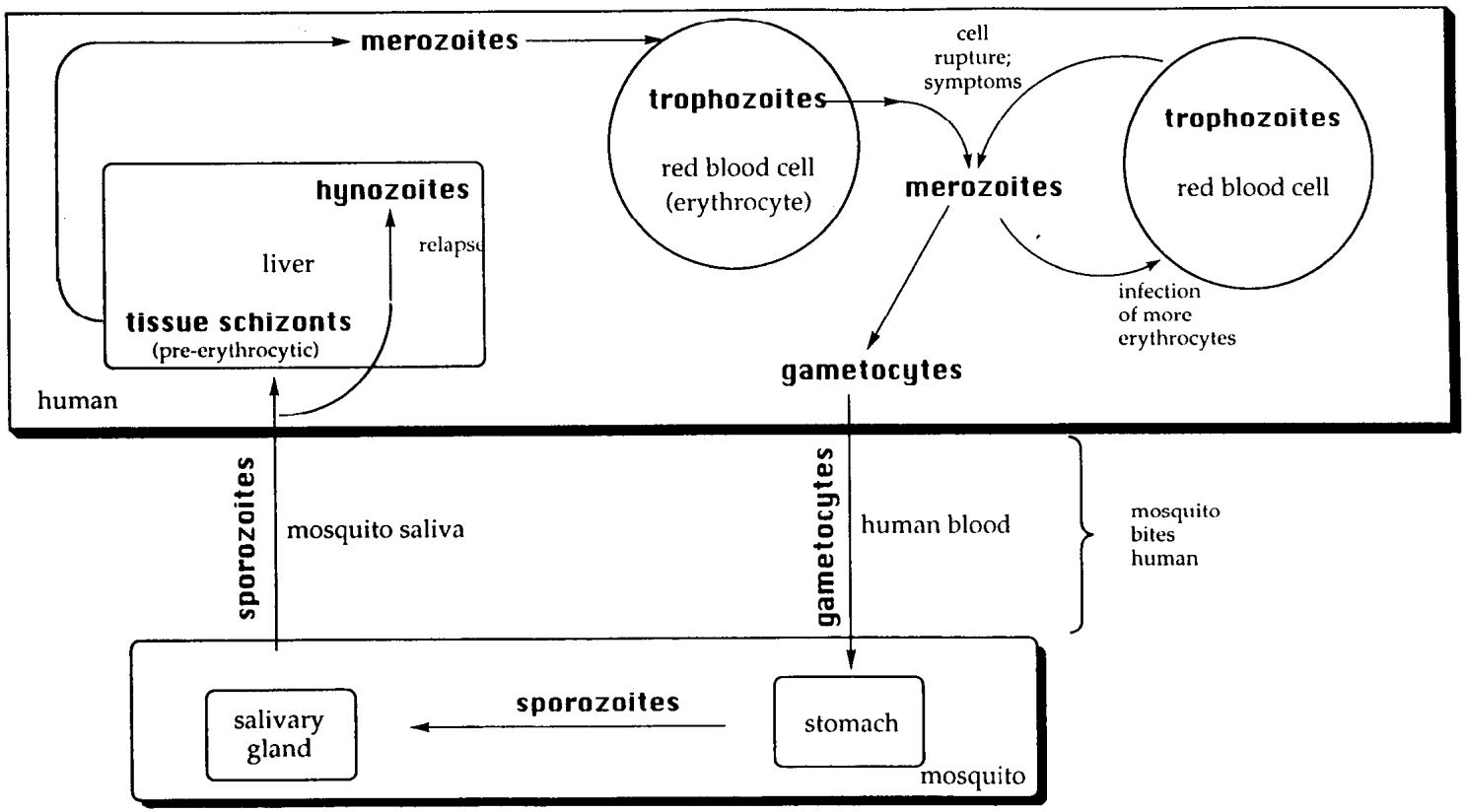

Fig. 59.1 Plasmodia life cycle.

symptoms that recur every second day; each erythrocytic cycle is completed within 41–45 hours. As a true relapsing malaria, it is characterized by a prolonged or secondary tissue development stage which can remain dormant for very long periods in the liver. Relapse can occur months or even years after clearance of the initial blood-stage infections. This allows vivax to remain endemic in areas that experience cold winters with no chance for transmission by mosquito. Treatment with drugs that kill only the erythrocytic forms of the parasite will not effect a radical cure of a vivax infection. Vivax is widely spread geographically with the notable exception of tropical Africa. This has been explained by the absence of a red cell surface antigen in most black Africans that vivax requires for cell penetration (10). *Plasmodium ovale* is much less widespread than the other three species, with patchy distributions where it does occur in tropical Africa. A relapsing malaria, its features are similar to that of vivax with an erythrocytic cycle of 49–50 hours. Ovale and vivax overlap very little geographically. *P. vivax* and *P. ovale* infect only young erythrocytes. *Plasmodium malariae* infections or quartan malaria recurs every third day or 72 hours and is noted for persistent reappearance of symptoms. Even so, it is not a true relapsing malaria; *P. malariae* has extremely long-lasting erythrocytic forms which can persist in an infected host for decades at very low parasite densities. Mature red blood cells are the target of *P. malariae* infection. Its geographic distribution is broad but irregular.

The three species of malaria above cause a comparatively mild form of the disease. Given the selectivities for age of the erythrocyte infected, the degree of total parasitemia is limited. Red cells are destroyed in the peripheral capillaries and anemia results. Although in nonimmune children and travelers, these symptoms can be quite severe, the global incidence of mortality from vivax, ovale, and malariae is extremely small. The possibility of relapse or deep recrudescence, however, makes the treatment and monitoring of patients critical.

In contrast, *Plasmodium falciparum,* subtertian malaria, or tropical malaria can lead to serious and life-threatening conditions when untreated. Erythrocytes of all ages can become infected by *P. falciparum* and thus, a high percentage of red cells can become parasitized. Infection of erythrocytes causes them to become sticky which allows them to bind to endothelial cell membranes and to clump together in the deep organs. Microcirculatory arrest occurs and when this happens in the brain, the condition is termed cerebral malaria: delirium, coma, convulsion, and death may ensue. Falciparum malaria is characterized by erythrocytic cycles of 48 hours but it does not relapse since it forms no hypnozoites in the liver. It is the most serious form of the infection, most widespread geographically, and accounts for the vast majority of malaria deaths. In areas of intense transmission, persons may be infected by more than one of these species at a time, causing complications in treatment.

Humans are not the only creatures that are parasitized by Plasmodia species. Several other parasites and their hosts have been used extensively for research in malaria. *Plasmodium berghei, P. vinckei,* and *P. yoelii* are useful models for malaria in mice and rats while *P. cynomolgi* and *P. knowlesi* are studied in various monkey species. The human parasites can be studied in owl monkey (aotus) and in splenectomized chimpanzee. Many avian and reptilian malarias are known but their value as experimental models of human malaria is not as great as the rodent and primate malarias.

The most common method for evaluating the antimalarial activity of drugs and experimental compounds is the microdilution technique introduced by Desjardins and coworkers in 1979 (11) and modified by Milhous (12). Cultured intraerythrocytic asexual forms of *P. falciparum* are treated with serial dilutions of compounds to be tested. Inhibition of uptake of [G-^{3}H]hypoxanthine by the

parasites serves as the indicator of antimalarial activity. [2,8-^{3}H]Adenosine may also be used as the radiolabel in these assays (13). For some types of studies, standard cultures are often unsuitable due to the high proportion of uninfected erythrocytes. A new procedure for producing highly concentrated cultures of *P. falciparum* from the ring stage provides a new tool in malaria research (14).

1.3 Parasite Biochemistry

The biology and biochemistry of Plasmodia are topics of wide interest. The information from those fields that more directly impacts on the design of new antimalarial agents is presented briefly in the following.

For the majority of their life-cycle in humans, malaria parasites live in red blood cells. Within the erythrocytes, the parasites feed on hemoglobin, digesting the protein and releasing the heme. Hemoglobin digestion by *P. falciparum* proceeds by an ordered metabolic pathway. The initial events are the endocytosis of hemoglobin from the host cytoplasm and transport to the food vacuole where the protein portion (globin) is degraded by a series of proteolytic enzymes. The heme, which is released as a by-product of hemoglobin degradation as ferriprotoporphyin IX (FP), cannot be metabolized by the parasite. Hemin is toxic to most biological systems due to its ability to generate active oxygen species from molecular oxygen. In most organisms, hemin is degraded into bile pigments; plasmodia, instead, detoxified hemin by the activity of a heme polymerase which converts FP into β-hematin polymers (15). The polymer linkage involves a covalent bond between the iron atom of one heme and the propionyl side chain of another (16). The β-hematin is nontoxic due to insolubility in water at physiologic or acidic pH values and accounts for the dark brown color of malaria pigment or hemozoin. A reduction in the activity of the heme polymerase would be expected to cause a build-up of precursors, such as FP, partially degraded hemoglobin, and ingested hemoglobin.

Malaria parasites evade the human immune system through a process of continuous variation in a specific protein, erythrocyte membrane protein 1 (EMP-1) (17–20). Upon infection of the red blood cell, *P. falciparum* synthesizes EMP-1 which presents on the surface of the infected cell. EMP-1 serves to bind infected cells to blood vessels in the brain and in other organs. The presence of the EMP-1 protein would also be expected to notify the immune system of an infectious agent present in the cell. But, the parasite carries as many as 150 genes for EMP-1, each encoding a slightly different protein. New variants of EMP-1 allow the parasite to avoid destruction by immune processes. Studies have estimated that about one in 50 of each new generation of parasite secretes a different EMP-1 protein.

Plasmodia synthesize dihydrofolate by a pathway unique to micro-organisms. Para-aminobenzoic acid (PABA) is linked with a pteridine to form dihydropteroate by the enzyme dihydropteroate synthetase (DHPS), an enzyme not present in mammals. Then, conjugation of dihydropteroate with glutamate forms dihydrofolate (dihydropteroylglutamate). In contrast, mammalian cells obtain dihydrofolate through reduction of dietary folic acid. Sulfonamides and sulfones, inhibitors of DHPS, are selectively toxic to the parasite and relatively safe in the human host.

Malaria parasites are unable to utilize preformed pyrimidines using "salvage pathways" as mammalian cells do. Rather, plasmodia synthesize pyrimidines de novo. An important enzymatic target is dihydroorotate dehydrogenase (DHOD) which catalyzes the conversion of dihydroorotate to orotate, an intermediate in the pyrimidine biosynthetic pathway. Some compounds with antimalarial action such as atovaquone have been found to be inhibitors of DHOD. In a step further along in pyrimidine biosynthesis, tetrahydrofolate is a required cofactor. Compounds

that inhibit dihydrofolate reductase (DHFR) effectively cut off the supply of tetrahydrofolate. Thus, compounds such as pyrimethamine and proguanil that inhibit DHFR are effective antimalarial agents.

Erythrocytes infected by plasmodia suffer oxidant damage from the parasite; the parasite causes measurable oxidation to the host red blood cell (21). The cell may be placed under oxidant stress from parasite-generated oxidants and from a weakening of the defense mechanisms of the cell itself. Increases in methemoglobin formation and lipid peroxidation have been documented in infected cells.

1.4 Global Incidence

Malaria is endemic in 91 countries with small pockets of transmission occurring in a further eight countries (data are for 1995). Although *Plasmodium falciparum* is the predominant parasite, falciparum malaria occurs only sporadically or does not exist in seven of those countries. For comparison, in 1955, there were 140 countries or areas where malaria was endemic. Global statistical information on malaria is available from the WHO web site (4). See Tables 59.1 and 59.2.

More than forty percent of the world's population, in excess of 2 billion people, live in areas where malaria is transmitted (e.g., parts of Africa, Asia, Central America, Caribbean, North America, Oceania, and South America). The 1992 world population of about 5,430 million people can be classified according to the status of malaria in their geographic areas as follows:

- Malaria-free areas (3,150 million people, or 58%). The disease has disappeared or was eliminated by organized antimalaria efforts, and the malaria-free status has been maintained in areas inhabited by 1,690 million people (31%). In areas with 1,460 million people (27%), malaria never existed or disappeared without specific antimalaria measures.
- Areas where endemic malaria had been considerably reduced or eliminated but transmission has now been reinstated, and/or areas where the situation is unstable or deteriorating (1,780 million people, or 33%). These areas include zones where severe malaria problems have developed following major ecological or social changes.
- Areas, mainly in tropical Africa, where endemic malaria is basically unchanged. Any existing control programs are in a planning or early implementation stage and have very limited resources (500 million people, or 9%).

Historically, malaria was endemic throughout much of the continental United

Table 59.1 Number of Malaria Cases Reported, by WHO Region (Thousands), 1983–1992[a]

WHO Region	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992[c]
Africa[b,c]	3,168	4,422	13,207	17,927	20,588	23,235	14,124	2,012	594	420
Americas	831	932	911	951	1,018	1,120	1,114	1,057	1,231	1,188
South-East Asia	2,731	3,003	2,501	2,685	2,834	2,789	2,957	2,960	3,044	3,092
Europe	73	62	57	47	28	25	21	13	16	22
Eastern Mediterranean	304	335	391	612	608	428	531	586	541	305
Western Pacific	842	1,410	1,177	1,307	1,145	1,002	1,071	1,032	968	733
Total (excl. Africa)	5,781	5,742	5,037	5,602	5,633	5,364	5,694	5,648	5,800	5,340

[a]The information provided does not cover the total population at risk in some instances. From Ref. 22
[b]Mainly clinically diagnosed cases.
[c]Incomplete figures.