

The importance of strict patient definitions in studies of malaria pathogenesis

Most clinical studies of malaria pathogenesis in humans are performed as cross-sectional studies comparing different patient categories. We have looked at studies of similar clinical manifestations of

malaria and found wide variation in their conclusions. The differences could present true geographical variation caused either by transmission pattern or by host and parasite genetics, or both. Another important reason for the variation is, however, ill-defined patient groups that have not been designed specifically to address the questions raised by the studies.

The bulk of evidence suggests that the different complications of malaria differ in pathogenesis, management and prognosis. Malarial anaemia and cerebral malaria affect different age groups of children

exposed to different levels of malaria transmission¹ and are associated with different cytokine profiles². In fact, probably both malarial anaemia and cerebral malaria should be further subdivided into distinct entities^{3,4} (B.A. Astrup *et al.*, unpublished). We thus propose that severe malaria should not be studied as one disease entity, and that careful and uniform case definitions are crucial for studies of malaria pathogenesis. It is also problematic to use so-called 'mild' malaria as controls. Mild malaria usually denotes patients with short symptom

Box 1. Guidelines for the design of cross-sectional studies of malaria pathogenesis^a

It must be malaria

(1) Malaria parasites must be evident

Asymptomatic infections are common in endemic areas

- Define cut-off level for parasitaemia above usual asymptomatic infections.

Some complications, for example malarial anaemia, might occur late, even after parasite clearance

- Design studies longitudinally to follow progression from early stage with overt parasitaemia, or
- Use other parameters to show recent malaria infection, for example pigment or antigen detection.

(2) Characteristic febrile illness must be documented

Fever fluctuates and might be affected by antipyretic drugs

- Define fever as axillary temperature >37.5°C measured within 24 h of admission.

(3) Exclude other aetiologies to the findings

Asymptomatic malaria infections might coincide with other infections to mimic acute malaria

- Exclude common febrile illnesses, for example respiratory tract infection, gastro-enteritis, urinary tract infection, meningitis, etc.

Unrelated conditions might cause complications similar to those of severe malaria

- Exclude common causes of coma in studies of cerebral malaria, for example trauma, epilepsy, etc.
- Exclude common causes of anaemia in studies of malarial anaemia, for example nutritional deficiencies, haemoglobinopathies, bleeding, hook worm infestation, etc. (see below).

The studied syndrome must be well defined

(1) General

Severe malaria syndromes have different pathogenesis

- Avoid pooling patients into one group with 'severe malaria'.
- Certain indicators of severity, such as hyperparasitaemia or hyperpyrexia, do not induce a specific clinical picture*
- Restrict comparisons to syndromes that are likely to have uniform pathogenesis, or
 - Study the effect of such indicators in longitudinal studies.

(2) Cerebral malaria

Severity must be quantified in order to compare with other investigations

- Use standard coma scales, that is Blantyre coma scale for children and Glasgow coma scale for adults.

Patients in coma must be truly unrousable

- Allow 60 min after convulsions for the patient to regain consciousness and give diagnostic glucose bolus.

Several mechanisms might cause the syndrome cerebral malaria

- Improve case definitions by characterization of seizures (electroencephalogram if possible), measurement of cerebral blood flow (if possible), determination of acid-base status, spinal fluid investigations, etc.

(3) Malarial anaemia

Severity of the anaemia must be well defined

- Define severe anaemia as haemoglobin (Hb) <5 g dl⁻¹ and specify Hb of control groups.

In most malaria endemic areas, patients with confounding factors, such as nutritional deficiency and concomitant helminth infestations, cannot be excluded

- Omit 'grey zones' by comparing groups with Hb values that are separated by an interval of at least 1 g dl⁻¹.

Malarial anaemia might be associated with acute or chronic infection or cerebral malaria

- Attempt to group patients accordingly, or
- Follow patients longitudinally to identify factors associated with progression to anaemia.

Controls must be relevant to the hypothesis

(1) Control groups must be comparable with regard to parameters that are not studied

Lack of matching might result in comparison of incomparable patients, for example early and late stage

- Compare patients who have similar grades of parasitaemia, temperature, symptom duration, etc. and differ only in the studied clinical syndrome.

Too careful matching might hide true differences, for example age-dependent differences hidden by age matching

- Select meaningful criteria for matching.

^aText in italics denotes a problem followed by suggested solutions in roman.

duration and low parasitaemia. We find it more relevant to compare patients with similar degree of infection but with a different clinical outcome.

Guidelines for optimizing cross-sectional studies of malaria pathogenesis are given in Box 1. Our three main points are: (1) it must be malaria; (2) the clinical syndrome must be well defined; and (3) controls must be relevant to the raised hypothesis. In some cases, the scope of a study requires deviation from the guidelines. One example is studies of bacteraemia complicating *Plasmodium falciparum* malaria, in which case, exclusion of other infections is obviously not applicable. In such cases, it is important to explain why standard definitions have not been followed and to provide the necessary clinical information to allow comparisons with other studies.

It is unfortunate that many studies have not adhered to these basic principles of patient selection. The most common reason is probably that acceptable sample sizes cannot be obtained within a specified time in most study sites. However, even if sample sizes become smaller, the use of a more specific read-out system is likely to give clearer results. Another reason is that laboratory scientists often receive clinical samples without direct involvement in the characterization of the patient groups. A close collaboration between clinical staff and biomedical scientists is essential from the planning through to the execution and publication of the studies. Finally, sponsors must be convinced of the importance of long-term funding, as the only way to obtain reliable results is by taking the necessary time.

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New trends in parasitism in Bulgaria

A rise of prevalence and incidence rates of parasitic diseases in Bulgaria during the past decade^{1–3} has led to an increase in their public health importance. The data presented here, regarding parasitic diseases between 1991–2000, were collected by the Departments of Medical Parasitology at District Hygiene Epidemiological Inspections (DHEI) and submitted for annual analysis to the Bulgarian Ministry of Health and the National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria.

Indigenous parasitic diseases

Indigenous parasitic diseases – zoonoses, such as hydatidosis – have increased in Bulgaria for the past ten years (Fig. 1). Between 1950 and 1962, a national programme for surveillance and control of hydatidosis resulted in a fall in its incidence (G. Genov, DSc thesis, Medical University of Sofia, 1974). However, since 1993, there has been a marked rise in morbidity resulting from hydatidosis. From 1991 to 2000, 5431 cases were registered by DHEI, of which 89.6% consisted of primary infections (4865 cases) and 10.4% resulted from postoperative relapses (566 cases). In some regions of Bulgaria, such as Sliven, Burgas, Pazardjik and Chaskovo, morbidity was much higher (15–27 per 100 000 people) than the country average over the same period (2.4–8.5 per 100 000 people). Of all patients in Bulgaria subjected to surgical treatment, 23% (1231 cases) were 0–19 years old^{1–5}.

Many factors contribute to the perpetuation and spread of the disease, such as an absence of a national control programme because of the financial problems of the country, the presence of

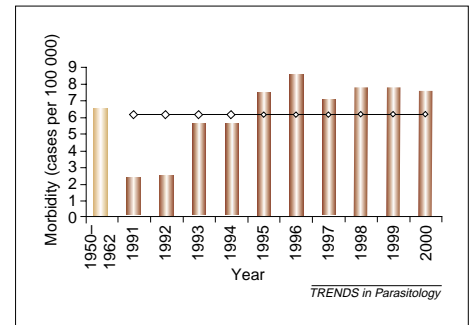


Fig. 1. Human hydatidosis morbidity in Bulgaria from 1991 to 2000.

large numbers of stray dogs and human habits, such as slaughterhouses permitting dogs access to infested viscera, feeding of dogs with condemned offal that harbours cysts and insufficient health education^{1–5}.

The situation urgently requires the revival of a national control programme.

From 1991 to 2000, there was a total of 96 outbreaks of trichinellosis, each of them involving 5–250 patients^{1–4,6}. This is a substantial rise compared with the 50-year period from 1922 to 1974, when 38 outbreaks were reported, involving 726 symptomatic patients that resulted in 17 fatalities (G. Genov, *op. cit.*). From 1991 to 2000, the number of infected people displaying mild to severe symptoms also increased to 1654 cases. Two out of 5683 individuals who consumed infected meat died. From 1987 to 1991, 97.4% of 38 trichinellosis outbreaks in humans were sourced to the wild boar. However, over the past ten years, the source of trichinellosis infection has been increasingly attributed to the domestic pig, which accounted for 54.2% of 96 outbreaks.

Incidence of *Taenia saginata* infections in Bulgaria can be classified as moderate, with rates of 0.9–1.4 per 100 000 people^{1–3}. Although, from 1991–2000, no human infections with *T. solium* were recorded, 1–3 cases of human cysticercosis are diagnosed each year. Seroprevalence of toxoplasmosis ranged from 35.2% to 42.2%. Over the same ten-year period, a few cases of cryptosporidiosis ($n = 1–3$), blastocystosis ($n = 5–20$) and visceral leishmaniasis ($n = 1–3$) were also recorded^{1–3}.

Imported parasitic diseases

Of the 27 parasitic diseases imported into Bulgaria, malaria has the greatest impact on health^{1–3,9,10}. Of a total of 382 malaria episodes registered during the period, 95.0% were imported into Bulgaria from countries where malaria is endemic, 4.7%