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**Mathematical Models of the Immunological and Clinical Epidemiology of
Plasmodium falciparum Malaria**

COORDINATOR

Eberhard-Karls-University Tübingen
Department of Medical Biometry
Westbahnhofstr. 55
72070 Tübingen
Germany

Prof. Dr. Dietz, Klaus
E-M: klaus.dietz@uni-tuebingen.de
TEL: +49 7071 2972112
FAX: +49 7071 295075

PARTNERS

Hospital Clinic, Barcelona
Unidad de Epidemiologia y Biostatistica
Villarroel 170
08036 Barcelona
Spain

Dr. Alonso, Pedro
E-M: alonso@medicina.ub.es
TEL: +34 3 227 5706
FAX: +34 3 451 5272

Università di Roma
Istituto di Parassitologia
Piazzale Aldo Moro
50085 Roma
Italy

Prof. Coluzzi, Mario
E-M: coluzzi@axrma.uniroma1.it
TEL: +39 06 4991 4932
FAX: +39 06 4991 4644

Institut Pasteur
Biomedical Parasitology
28, r. Dr. Roux
75015 Paris
France

Dr. Druilhe, Pierre
E-M: druilhe@pasteur.fr
TEL: +33 1 45 68 85 78
FAX: +33 1 4568 8640

London School of Hygiene and Tropical
Medicine
Department of Medical Parasitology
Keppel Street
London WC1E 7HT
England

Prof. Greenwood, Brian
E-M: b.greenwood@lshtm.ac.uk
TEL: +44 171 927 2348
FAX: +44 171 6368739

Wellcome Trust - Kenya Medical
Research Institute
P.O. Box 230
Kilifi
Kenya

Dr. Marsh, Kevin
E-M: kmarsh@africaonline.co.ke
TEL: +254 1252 2063
FAX: +254 1252 2390

University of Colombo
Malaria Research Unit
Department of Parasitology
P.O. Box 271, Kynsey Road
Colombo 8
Sri Lanka

Prof. Mendis, Kamini
E-M: mendisk@who.ch
TEL: +94 1 688660
FAX: +94 1 699284

Hôpital Albert Schweitzer
Laboratoire de Recherches
B.P. 118
Lambaréné
Gabon

Dr. Ndjave, Maryse
E-M: schweitzer@gab.healthnet.org
TEL: +241 581095
FAX: +241 581196

University of Oxford
Institute of Molecular Medicine
Headington
Oxford OX3 9DU
Great Britain

Prof. Newbold, Chris
E-M: cnewbold@hammer.imm.ox.ac.uk
TEL: +44 1865 222317
FAX: +44 1865222444

Institut Pasteur
Unité d'Immunologie Moléculaire des
Parasites
25, rue du Docteur Roux
75015 Paris
France

Dr. Puijalon, Odile
E-M: omp@pasteur.fr
TEL: +33 1 45688623
FAX: +33 1 40613185

Institut de Médecine Tropicale du Service
de Santé des Armées
Unité de Parasitologie
F-13007 Marseille
France

Dr. Rogier, Christophe
E-M: christophe.rogier@wanadoo.fr
TEL: +33 4 91152953
FAX: +33 4 91594477

Centre National de Lutte Contre le
Paludisme
01 BP 2208
Ouagadougou 01
Burkina Faso

Dr. Sirima, Bienvenu Sodiomou
E-M: dkatakou@bur.healthnet.org
TEL: +226 306655
FAX: +226 310477

Institut Pasteur de Dakar
Unité d'Épidémiologie
36, avenue Pasteur, B.P. 220
Dakar
Sénégal

Dr. Spiegel, André
E-M: spiegel@pasteur.sn
TEL: +221 23 51 81
FAX: +221 23 87 72

Swiss Tropical Institute
Department of Public Health &
Epidemiology
Socinstr. 57/P.O. Box
CH-4002 Basel
Switzerland

Dr. Smith, Tom
E-M: Thomas-A.Smith@unibas.ch
TEL: +41 61 2848273
FAX: +41 61 2717951

Institut Français de Recherche
Scientifique pour le
Développement en Coopération (ORSTOM)
213 Rue La Fayette
F-75480 Paris Cedex 10
France

Dr. Trape, Jean-François
E-M: trape@belair.orstom.sn
TEL: +33 221 320962
FAX: +33 221 321675

University of Edinburgh
Institute of Cell, Animal and Population
Biology
West Mains Road
Edinburgh EH9 3JT
Scotland

Ifakara Centre
National Institute of Medical Research
P.O.Box 53
Ifakara
Tanzania

Prof. Walliker, David
E-M: walliker@srv0.bio.ed.ac.uk
TEL: +44 131 650 5548
FAX: +44 1316673210

Dr. Kitua, Andrew
E-M: sysop@tan3.healthnet.org
TEL: +255 56 164
FAX: +255 56 3566

Mathematical models of the immunological and clinical epidemiology of *Plasmodium falciparum* malaria

Abstract

1. Objectives

The objectives of the project were to develop and validate mathematical models of *Plasmodium falciparum* (*P.f.*) malaria, appropriate for assisting in the planning and evaluation of malaria control and research.

2. Principal activities and methodologies used

2.1 Development and validation of models

The following activities were initiated sequentially, but pursued concurrently, with interactive feedbacks:

1. Critical review of published malaria models;
2. identification of relevant and accessible primary data sets and definition of data-based quantitative simulation targets;
3. model formulation and fitting of simulations to data-based targets;
- (d) sensitivity analyses, including implications for pathogenesis and control.

2.2 Meetings

Six meetings were organised for brainstorming: model assumptions; parameter values; clinical and epidemiological behaviour of *P.f.* malaria; appropriate primary data sets; critical review of work accomplished; orienting further work.

3. Major results obtained

New intra-host models of *P.f.* asexual parasitaemia and gametocytaemia were developed that are more realistic than previous models, both with respect to their biological assumptions and their behaviour. The realism of the new models makes them potentially useful tools to *assist* in planning and evaluation of malaria control, intervention trials, epidemiological research, research and development of new tools, especially vaccines. With respect to the new model of asexual parasitaemia, some potentially important implications concerning pathogenesis and expected effect of vaccines have been discussed at the AMVTN meeting in Accra and will be presented at the meeting “Variant proteins expressed on the surface of infected erythrocytes and their role in developing malaria vaccines”, convened by the US Navy Medical Research Unit.

Mathematical models of the immunological and clinical epidemiology of *Plasmodium falciparum* malaria

Summary of final report

1. Context and Objectives

1.1 Context

Malaria, especially *Plasmodium falciparum* (*P.f.*), continues to be one of the most important causes of human mortality and morbidity, and is a serious obstacle to the development of tropical countries. Malaria eradication programmes, based on insecticides and drugs, failed to achieve their goal. Research towards the development of *P.f.* vaccines is very active and it seems likely that effective vaccines will eventually emerge. One can predict that, for the majority of populations currently affected by *P.f.*, the addition of vaccination to the tools now available will not eradicate malaria but lead to a new balance between host and parasite populations. Hence the research aiming at a better knowledge and understanding of the natural history of the infection is relevant. Mathematical modelling can be useful as a component of such research. However, it was found that available models made inadequate biological assumptions, and that their behaviour was too unrealistic. This motivated the present Concerted Action.

1.1 Objectives

The objectives of this Concerted Action are to develop and validate better *P.f.* models, appropriate for *assisting* in

- (1) the planning and evaluation of malaria control programmes, vaccine trials, and investigations of the natural history of *P.f.*;
- (2) guiding decisions concerning investment in the development of new tools.

The approach to developing *better* models than those previously available is based on quantitatively rigorous comparison between model outputs and the best available data, critical review of literature, and sustained confrontation with expert advice concerning the biology and behaviour of *P.f.* malaria in individuals and in populations.

2. Activities

2.2 Development and validation of models

In the development of a given model, the following activities are initiated sequentially, but pursued concurrently, with interactive feedbacks:

- (a) Review and monitoring of the malaria (mainly *P.f.*) *literature*;
- (b) Identification of relevant and accessible *primary data sets*, and definition of *data-based quantitative simulation targets*; so far data sets provided by the USPHS (malariatherapy data) and by three of this project's partner institutions were used;
- (c) *model formulation, computer programming, and fitting of simulations to data-based targets*;
- (d) *sensitivity analyses, including implications for pathogenesis and control.*

2.2 Internal meetings

Six internal meetings, two plenary, four partial, were organised for brainstorming concerning all aspects of the work, including in particular: (i) biology-based model assumptions; (ii) parameter values; (iii) clinical and epidemiological behaviour of *P.f.* malaria; (iv) identification and accessibility of appropriate primary data sets; (v) critical review of work accomplished; (vi) orienting further work.

2.3 Presentations to conferences

Presentations of results were made (will be made, respectively) at the following conferences:

- (a) Rome: Centenary Malaria conference, November 16-19, 1998;
- (b) Heidelberg: 7th Malaria EC Contract Holders Meeting, September 27-29, 1999; (c) Accra, October 24-27, 2000: Meeting of the AMVTN (African Malaria Vaccine Testing Network).
- (d) Bethesda, February 27-28, 2001: Meeting "Variant proteins expressed on the surface of infected erythrocytes and their role in developing malaria vaccines", convened by the US Navy Medical Research Unit.

2.4. Transfer of technology

Help was provided in the teaching of the “Ecole Mathématiques et Malaria” (Yaoundé, September 4-15, 2000), an international workshop organised by a team of African mathematicians, with cooperation from France.

2.5 Role of partners

(a) There were 16 partner institutes, 9 in Europe, 7 in DC's.

(b) The actual modelling work presented in this report was performed in the department of the Medical Biometry, University of Tübingen.

(c) The partners, through meetings and additional contacts, have made essential contributions to the modelling.

(d) In addition, the partners have pursued their own research programmes, including the production of new relevant data on malaria.

3. Results achieved

3.1 Review of previous intra-host models of malaria (see Publication 1)

General conclusions

Intra-host malaria models provide a strong stimulus and a useful framework for the scientific debate concerning the natural history and the control of malaria.

The available models raise several serious questions concerning both their assumptions and their behaviour. While there is no certainty about how ‘realistic’ a model should be, even to address a clearly defined question, an effort towards greater realism is probably to be encouraged.

Among amendments worth exploring, the following may be of general value:

- (i) a more rigorous quantitative comparison between model outputs and ‘reality’;
- (ii) a systematic investigation of *combinations* of types of parasite diversity and density regulation mechanisms; (iii) let some parameters vary among individual hosts; (iv) replace continuous-time modelling by discrete-time modelling.

*3.2 A new model of intra-host *P.f.* asexual parasitaemia*

3.2.1 Description and validation of the model (see Publication 3)

a) Abstract

A new mathematical model of *P. falciparum* asexual parasitaemia is formulated and fitted to 35 malariatherapy cases making a spontaneous recovery after primary inoculation. Observed and simulated case-histories are compared, with respect to 9 descriptive

statistics. The simulated courses of parasitaemia are more realistic than any previously published. The model uses a discrete time step of two days. Its realistic behaviour was obtained by the following combination of features: (i) intra-clonal antigenic variation; (ii) large variation of the variants' baseline growth rate, depending on both variant *and* case; (iii) innate autoregulation of the asexual parasite density, variable among cases; (iv) acquired variant-specific immunity; (v) acquired variant-transcending immunity, variable among cases. Aspects of the model's internal behaviour, concerning variant dynamics, as well as the respective contributions of the three control mechanisms (iii) to (v), are displayed. Some implications for pathogenesis and control are discussed.

(b) *Remarks*

(i) All the model's assumptions are biology-based. However, model formulations have to be more clearcut than current biological knowledge. The simulations, by making the model's internal behaviour explicit, open it to challenge (and amendment) by new biological data.

(ii) The model's most problematic biological assumption concerns variant expression. The parasite was assumed to adapt variant expression to the changing immunological environment early in the course of schizogony, i.e. to anticipate immuno-selection. The existence of the phenomenon is suggested by data from *P. knowlesi*, but it would require an as yet unknown mechanism.

3.2.2 Sensitivity analysis and amendment of the model (see Publication 6)

An improved method of fitting, including hypercube sampling and minimisation, was applied. Alternative assumptions were tested. The need for the five features producing realistic behaviour was confirmed. The adaptation of variant-expression to the changing immunological environment was abandoned. A distinction between primary and secondary variant-specific immune responses was introduced. More realistic simulation of parasitaemia was obtained with the amended model, which has probably a more solid biological basis.

3.3. A new model of gametocytaemia

3.3.1 Development and comparative evaluation of seven alternative models, and selection of the best model (see Publication 2)

Abstract

In this paper, the transition of asexual blood stages of *P. falciparum* to gametocytes is investigated. The study is based on daily data. Several mathematical models are fitted with maximum likelihood and compared. The models differ in the assumptions made. Gametocyte mortality is modelled as being (i) constant over time, (ii) linearly increasing over time, (iii) linearly increasing over gametocyte age, and (iv) exponentially increasing over gametocyte age, respectively. The transition rate is either kept constant per patient or piecewise constant within intervals that correspond to waves of asexual parasitaemia. According to likelihood ratio tests, the models with age-dependent mortality rate and wave-dependent transition rates are superior to the models with constant transition rate and/or constant or time-dependent mortality rate. The best fits are reached for models with exponentially increasing (Gompertz-type) mortality. Furthermore, an impact of high asexual parasite densities on the survival of gametocytes, interpreted as cytokine-mediated effect, is evident in some cases.

3.3.2 Use of the best model to estimate conversion probability and the sequestration and circulation times of *P.f.* gametocytes (see Publication 4)

(a) *Abstract*

Part of the parasite's life cycle is spent in man's blood, mainly as asexual stages. A fraction of the asexual parasites develops into gametocytes (gamete precursors) while sequestered in deep tissues. After re-entering the circulation, gametocytes can be picked up by a mosquito to continue the parasite's life cycle. The conversion probability from asexual parasites to circulating gametocytes and of the gametocytes' sequestration and circulation times are estimated by fitting the dynamic model identified as best in the previous paper. The most remarkable findings are the large individual variation of conversion probabilities and circulation times, the average gametocyte circulation time of more than twice the currently accepted value, and the large variation of conversion probability among successive waves of asexual parasitaemia without any particular time pattern. The latter finding could be explained by an association between conversion probability and variation of PfEMP1.

(b) Remarks

(i) The large and unordered variation in gametocytogenesis among successive waves of asexual parasitaemia cannot be explained on the basis of any of the factors advocated in the literature (mainly on the basis of *in vitro* experiments) as affecting the conversion of asexual parasites to gametocytes.

(ii) That variation should be taken into account in the thinking concerning the natural history of gametocytogenesis (including its evolution) and the control of transmission (including the potential use of vaccines, or even the genetic manipulation of gametocytogenesis).

3.4 Analysis of re-inoculation data (see Publication 5)

(a) Abstract

Malariatherapy reinoculation data were examined to detect effects attributable to individual host-specific innate factors, through correlation between descriptive variables of first and second infections. Such an effect was demonstrated with respect to the first local maximum of the asexual parasite density. The effect was seen between two *Plasmodium falciparum* infections, homologous or heterologous but significant only for homologous re-inoculations. The effect was also seen – and significant - between *P.f.* and *P.ovale*, but not between *P.f.* and *P.malariae*. The data were also examined for systematic changes from first to second *P.f.* infection, as indicators of acquired immunity. The findings suggest the independent development of antiparasitic and antitoxic immunities.

(b) Remarks

This work is very relevant for modelling: (i) it supports the existence of a strong individual (presumably genetic) component of the innate immune response, as postulated in the new model of asexual parasitaemia; (ii) it provides important quantitative information concerning the development and persistence of antiparasitic and antitoxic immune responses.

3.5 Work on clinical data from Gabon

The main objective was to estimate the size and dynamics of the sequestered *P.f.* population on the basis of detailed clinical and parasitological follow-up data obtained in the course of treatment of uncomplicated malaria in partially immune children. A model was formulated and successfully fitted to the data. The data proved however insufficient to separate the estimation of key parameters. Two of the partner institutes are engaged in collecting clinical data including additional measurements likely to allow greater

discrimination in parameter estimation. Another partner is also engaged in modelling the sequestered parasite population.

3.6 Work on molecular (PCR-based) epidemiological data from Senegal and Tanzania

Various additional analyses of the two data sets, in cooperation with the respective partners were performed. The epidemiological interpretation of PCR-genotyping data and various approaches to modelling endemic malaria including multiplicity and diversity of inoculations have been discussed at the last meetings. Tentative simulation targets and a tentative simulation model were formulated.

4. Publications and papers

4.1 Publications

- (1) Molineaux L, Dietz K (1999) Review of intra-host models of malaria. *Parassitologia* 41: 221-231
- (2) Diebner HH, Eichner M, Molineaux L, Collins WE, Jeffery GM, Dietz K (2000) Modelling the transition of asexual blood stages of *Plasmodium falciparum* to gametocytes. *J. Theor. Biol* 202: 113-127
- (3) Molineaux L, Diebner HH, Eichner M, Collins WE, Jeffery GM, Dietz K (2001) *Plasmodium falciparum* parasitaemia described by a new mathematical model. (to appear in *Parasitology*)
- (4) Eichner M, Diebner HH, Molineaux L, Collins WE, Jeffery GM, Dietz K (2001) Genesis, sequestration and survival of *Plasmodium falciparum* gametocytes. Parameter estimates from fitting a model to malariatherapy data (submitted)
- (5) Molineaux L, Träuble M, Collins WE, Jeffery GM, Dietz K (2001) Demonstration of an individual host-specific factor in malariatherapy re-inoculation data (submitted)
- (6) Dietz K, Träuble M, Collins WE, Jeffery GM, Molineaux L (2001). Sensitivity analysis and amendment of a new mathematical model of *Plasmodium falciparum* parasitaemia (to be submitted after final revision)

4.2 Documents available on the Web

A complete compilation of all 262 individual gametocytaemia fits (see above, Publication (2)) is available on the internet web page

http://www.uni-tuebingen.de/biometry/eu/eu_index.html

5. Conclusion

Intra-host models of *P. falciparum* asexual parasitaemia and gametocytaemia have been developed that are more realistic than previous models, both with respect to their biological assumptions and with respect to their behaviour. The key elements of the approach were a critical review of the literature, the selection of appropriate primary data, the definition of precise quantitative simulation targets, a rigorous comparison between model simulations and targets, and sustained interaction with frontline investigators in the relevant disciplines. While no model, at least in biology, should ever be considered *definitive*, the new models' realism makes them potentially useful tools to *assist* in planning and evaluation of malaria control, intervention trials, epidemiological research, research and development of new tools, especially vaccines. With respect of the new model of asexual parasitaemia, some potentially important implications concerning pathogenesis and expected effect of vaccines were discussed at the AMVTN meeting in Accra and will be presented at the meeting "Variant proteins expressed on the surface of infected erythrocytes and their role in developing malaria vaccines", convened by the US Navy Medical Research Unit, and to be held in Bethesda on February 27-28, 2001.

In addition to results actually achieved, promising work on the estimation of the population of sequestered parasites and on molecular epidemiology of multiple and genetically diverse inoculations were initiated.

Mathematical models of the immunological and clinical epidemiology of *Plasmodium falciparum* malaria

Consolidated scientific report

2. Context and Objectives

2.1 Context

Malaria continues to be one of the most important causes of human mortality and morbidity. Among the four species of malaria parasites infecting man, *Plasmodium falciparum* (*P.f.*) causes the vast majority of malaria-associated deaths, and shares responsibility with *P. vivax* for most of malaria-associated disease. *P.f.* is prevalent in tropical developing countries, and is indeed an important obstacle to their development.

While malaria eradication programmes, largely based on insecticides and antimalarial drugs, yielded major benefits in terms of public health, global eradication failed. It is generally accepted that the causes of this setback are multiple, yet there has been a tendency to concentrate on inadequate implementation of the eradication strategy (e.g. on dwindling resources in the face of increasing costs, or failure to achieve or sustain adequate coverage) and on the blunting of the two main weapons used (through insecticide-resistance in the vector and drug-resistance in the parasite). There has been less recognition of intrinsic weaknesses of the eradication strategy. The latter include at least two major conceptual flaws, both leading to unrealistic optimism: (1) a serious underestimation of the oversaturation associated with intense transmission, as pointed out, with the help of a mathematical model, by Moshkovskij¹; (2) the implicit assumptions of uniform resting behaviour of vectors (see Molineaux et al.²) and of uniform participation of humans in mass-drug administration (see Molineaux & Gramiccia³).

Research towards the development of *P.f.* vaccines is very active in some laboratories in a number of countries, including several in the EU. The EC helps funding this research, and is also funding two related networks, namely VINCOMAL and its successor EMVI (aiming at the coordination of basic vaccine development research) and AMVTN (**A**frican **M**alaria

¹ *Bull. WHO*, **36**: 992-996, 1967

² *Bull. WHO*, **57**: 265-274, 1979

³ Molineaux, L. & Gramiccia, G. *The Garki Project*, WHO, Geneva, 1980

Vaccine Testing Network). Malaria vaccine research is intimately intertwined with research on the molecular biology of the parasite. A recent overview is available in the October 2000 issue of *Parasitology Today*, based on the *Molecular Approaches to Malaria* Conference, Lorne, Australia, 2-5 February 2000.

It seems likely that effective *P.f.* vaccines will be developed. However, the prediction that *P.f.* vaccination, at an achievable coverage, is likely to lead to eradication (see Gupta et al.⁴) is unrealistically optimistic. It is based on the assumption that a local population of malaria parasites is composed of "strains" which do not viably recombine to any significant extent. The frequency of viable recombination is however becoming increasingly obvious. Furthermore the prediction cannot be reconciled with the failure to interrupt transmission in several well documented trials based on vector control (see Dye et al.⁵; Molineaux & Gramiccia³). It is much more likely that, for the majority of populations currently affected by *P.f.*, the addition of vaccination to the tools now available will lead to a new balance between host and parasite populations. Hence the research aiming at a better knowledge and understanding of the natural history of the infection is relevant. Mathematical modelling has great potential usefulness as a component of such research. Indeed, ignoring explicit modelling commonly implies the uncritical adoption of potentially flawed implicit models. Available mathematical models were however inadequately critical about their biological assumptions and about their unrealistic behaviour. Hence the present project.

2.2 Objectives

The overall long-term objectives of this line of research are to develop and validate better *P.f.* models, appropriate for assisting in the planning and evaluation of malaria control programmes and field intervention trials, in particular vaccine trials, in investigating the natural history of *P.f.*, and in guiding decisions concerning investment in the development of new tools.

The specific objectives of the present project were to develop and validate some of the relevant models (see Activities, Results, Publications), to begin exploring implications for pathogenesis and control, in particular through vaccines, and to promote these modelling activities to assist in malaria control.

⁴ *Science*, **263**: 961-963, 1994

⁵ *Parasitology Today*, **12**: 88-89, 1996

The approach to developing *better* models than those previously available is based on quantitatively rigorous comparisons between model outputs and the best available data, on a critical review of the literature, and on the sustained confrontation with expert advice concerning the biology and behaviour of *P.f.* malaria in individuals and populations.

3. Activities

3.1 Development and validation of models

In the development of a given model, the activities listed below ((a) to (d)) are initiated sequentially, but pursued concurrently, with continuous interactive feedbacks. For only one of the models (the model of asexual *P.f.* parasitaemia in the individual host) was the exploration of implications for pathogenesis and control initiated. The stage reached in the work on different models and the corresponding activities are identified in Section 3 (Results achieved). Here the different kinds of activities undertaken are outlined and some of the key methods used are identified.

(a) Review and monitoring of the malaria (mainly *P.f.*) *literature*, including biology, immunology, epidemiology, control, vaccine development.

(b) Identification of relevant and accessible *primary data sets*, their processing as required (computerisation via scanning of typed lists and reformatting of computerised data) and definition of *data-based quantitative simulation targets*.

The following data sets (provided by the USPHS and by three of this project's partner institutions) have been processed so far:

- USPHS malariatherapy data (provided by Dr. WE Collins)
- clinical data from Gabon (Hôpital Albert Schweitzer, Lambaréné)
- molecular epidemiology data collected from pregnant women by Dr. D Schleiermacher (Institut Pasteur de Dakar and Institut Pasteur, Unité d'Immunologie Moléculaire des Parasites)
- molecular epidemiology data from a trial of insecticide treated bednets in Kiberege, Tanzania (Ifakara Centre and Swiss Tropical Institute)

(c) *Model formulation, and fitting of simulations to data-based targets.*

The models are formulated as difference equations. Parameter values are based on the literature, on expert advice and fitting. Fitting was done by informal trial-and-error, by hypercube sampling and minimisation. Goodness-of-fit was assessed by paired t-tests. The methods used are fully specified in the resulting publications.

(d) *Sensitivity analysis, including implications for pathogenesis and control.*

If a model produces realistic simulations (in comparison with the simulation targets), the following are explored: (i) the sensitivity of the model's realistic behaviour to changes in assumptions or parameter values; (ii) the implications in terms of pathogenesis and of the expected effects of interventions (including hypothetical interventions, in particular malaria vaccines).

3.2 Internal meetings

Internal meetings were organised for brainstorming concerning all aspects of the work, including in particular: (i) biological model assumptions; (ii) parameter values; (iii) clinical and epidemiological behaviour of *P.f.* malaria; (iv) identification and accessibility of appropriate primary data sets; (v) critical review of work accomplished; (vi) orienting further work.

Two kinds of meetings have been organised: (i) plenary meetings, addressing the project as a whole, to which all partner institutes were invited; (ii) sub-group meetings, addressing more specific issues, to which different subsets of partner institutes were invited. Participating institutes could be represented by more than one scientist at a given meeting. Some other scientists (from outside the partner institutes) were invited to some of the meetings.

There were two plenary meetings (Tübingen, February 4-6, 1998; Rome, November 20, 1998) and four sub-group meetings (Heidelberg, September 27-29, 1999; Tübingen, December 17-18, 1999, May 26-27, 2000, and October 6-7, 2000). Reports from the first four meetings have been included in the progress reports; reports from the last two meetings are attached to this final report.

1. Presentations to conferences

Presentations of the results were made (or will be made, respectively) at the following conferences:

(a) Rome, November 16-19, 1998: Centenary Malaria conference.

(b) Heidelberg, September 27-29, 1999: 7th Malaria EC Contract Holders Meeting.

(c) Accra, October 24-27, 2000: Meeting of the AMVTN. Interaction with that network was of particular interest, as it is also funded by the EC, and an important potential user of the results. The presentation, made by Prof. Dietz and attended, in addition to the AMVTN, by

staff and students of the Noguchi Institute, was received with great interest and followed by a long lively discussion.

(d) Bethesda, February 27-28 2001: Meeting “Variant proteins expressed on the surface of infected erythrocytes and their role in developing malaria vaccines”, convened by the US Navy Medical Research Unit

2. Transfer of technology

We received an invitation from a team of African mathematicians (headed by Dr. Bitjong Djombol, Yaoundé), in cooperation with Dr. Gauthier Sallet (Metz) to help in the teaching at the “Ecole Mathématiques et Malaria” (Yaoundé, 4-15 September 2000), an international workshop for African mathematics students interested in the practical application of mathematics (including statistics and modelling) to the malaria problem in Africa. A member of Prof. Dietz’s team (Dr. Martin Eichner), and one of the project’s partners (Dr. Christophe Rogier) did indeed teach in the workshop. The organisers were happy about their contribution, considered the workshop as a success, and plan to transform it into a regular activity.

2.5 Role of partners

(a) The actual modelling work presented in this report (and funded by the EC) was performed in the department of the coordinator (Medical Biometry, University of Tübingen).

(b) The partners were selected because of the quality and relevance of their past and current work, and of their willingness to contribute suggestions, critical comments and primary data. Through their participation in meetings, and through additional ad-hoc contacts, they have indeed made essential contributions to the modelling effort (see above, 2.1(b) and 2.2).

(c) In addition, the partners have pursued, on their own funding, their own research programmes, including the production of new data on biological, clinical and epidemiological aspects of *P.f.* malaria, contributions to the development of *P.f.* vaccines, and modelling various components of the problem. References to the partner’s publications are given in the section on partner final reports.

(d) As the focus of work moved from the USPHS malariatherapy data to data provided by the partners, their closer involvement in the Tübingen-based modelling work developed. This has started with Christophe Rogier and Tom Smith with respect to the data from Senegal and Tanzania (see Section 2.1(b)).

(e) In the course of the project, the EC has expressed concern about the small contribution of DC scientists to this Concerted Action. Out of 16 partner institutes, 6 are located in DC's. All of those make their own genuine contributions to malaria research. In four of them, the team leaders are nationals. The latter have mostly been unable to attend this project's meetings, because of competing tasks:

Dr. Kitua has been promoted to a job involving much more administrative work; Dr. Mendis took up a full-time job in WHO's Roll Back Malaria programme; Dr. Sirima is also responsible for his country's malaria control programme. In consultation with Dr. Mamadou Traoré, it was tried to make up, at least to some extent, for this lack of involvement of DC's, through the meeting with AMVTN (on the project's budget) and through participation in the Yaoundé workshop (on the organisers' budget).

3. Results achieved

3.1 Review of previous intra-host models of malaria (see Publication 1)

(a) Abstract

Intra-host models of malaria (and some related models of trypanosomiasis) are reviewed. A first section gives a short description of the different models, their purposes and the authors' conclusions. A second section discusses some common issues, including intra-host populations, the intra-host basic reproduction number (R_0) and growth rates, density regulation mechanisms (including acquired immunity), and the models' behaviour compared to that of *Plasmodium falciparum* in man.

(b) General conclusions

Intra-host malaria models provide a strong stimulus and a useful framework for the scientific debate concerning the natural history and the control of malaria.

The available models raise several serious questions concerning both their assumptions and their behaviour. While there is no certainty about how 'realistic' a model should be, even to address a clearly defined question, an effort towards greater realism is to be encouraged.

Among amendments worth exploring, the following may be of general value: (i) a more rigorous quantitative comparison between model outputs and 'reality'; (ii) a systematic investigation of *combinations* of types of parasite diversity and density regulation mechanisms; (iii) the variation of some parameters among individual hosts; (iv) replacing continuous-time modelling by discrete-time modelling.

(c) Two examples of the limitations of previous work

(i) The pioneering model of Anderson et al.⁶ is still the most influential. Saul⁷ showed that it allows unrealistically high growth rates. Gravenor & Lloyd⁸ replied that this was compensated by reducing the (postulated) fraction of successful merozoites. However, that very compensation limits the model's predictive value: it would lead to gross underestimation of the minimum efficacy required from a merozoite vaccine for controlling the growth of the parasite population.

(ii) Only McKenzie & Bossert⁹ set *a priori* quantified simulation targets. These are however defined so broadly that they do not allow the rejection of any of the authors' nine (rather different) models.

3.2 A new model of intra-host *P.f.* asexual parasitaemia

3.2.1 Description and validation of the model (see Publication 3)

a) Abstract

A new mathematical model of *P.f.* asexual parasitaemia is formulated and fitted to 35 malariatherapy cases making a spontaneous recovery after primary inoculation. Observed and simulated case-histories are compared, with respect to 9 descriptive statistics. The simulated courses of parasitaemia are more realistic than any previously published. The model uses a discrete time step of two days. Its realistic behaviour was obtained by the following combination of features:

(i) intra-clonal antigenic variation; (ii) large variation of the variants' baseline growth rate, depending on both variant *and* case; (iii) innate autoregulation of the asexual parasite density, variable among cases; (iv) acquired variant-specific immunity; (v) acquired variant-transcending immunity, variable among cases. Aspects of the model's internal behaviour, concerning variant dynamics, as well as the respective contributions of the three control mechanisms (iii) to (v), are displayed. Some implications for pathogenesis and control are discussed.

(b) Remarks

(i) All the model's assumptions are based on biological knowledge. However, model formulations have to be more clearcut than presently supported by evidence, and the data, while irreplaceable for the natural history of parasitaemia in individual hosts, were

⁶ *Parasitology*, **99**: S59-S79, 1989

⁷ *Parasitology*, **117**: 405-407, 1998

⁸ *Parasitology*, **117**: 409-410, 1998

⁹ *J. Theor. Biol.*, **188**: 127-140, 1997

collected before the development of molecular biology. The simulations, by making the model's internal behaviour explicit, open it to challenge by new biological data.

(ii) The model's most problematic biological assumption concerns variant expression. In the search for realistic output parasitaemia, the parasite was initially allowed to adapt variant expression to the changing immunological environment early in the course of schizogony, i.e. to anticipate immuno-selection. The existence of the phenomenon is suggested by data from *P. knowlesi*, but it would require an as yet unknown mechanism. (In the subsequent sensitivity analysis this model feature was removed.)

(c) *Selected results*

Observed and simulated individual courses of asexual parasitaemia are compared in Figure 1. The three cases selected had, respectively, the smallest, median, and largest number of local maxima (waves of asexual parasitaemia). Two model parameters are estimated from each individual case-history: the parasite density at which the innate immune response reaches half its maximum efficacy is proportional to the observed first local maximum density; the corresponding density

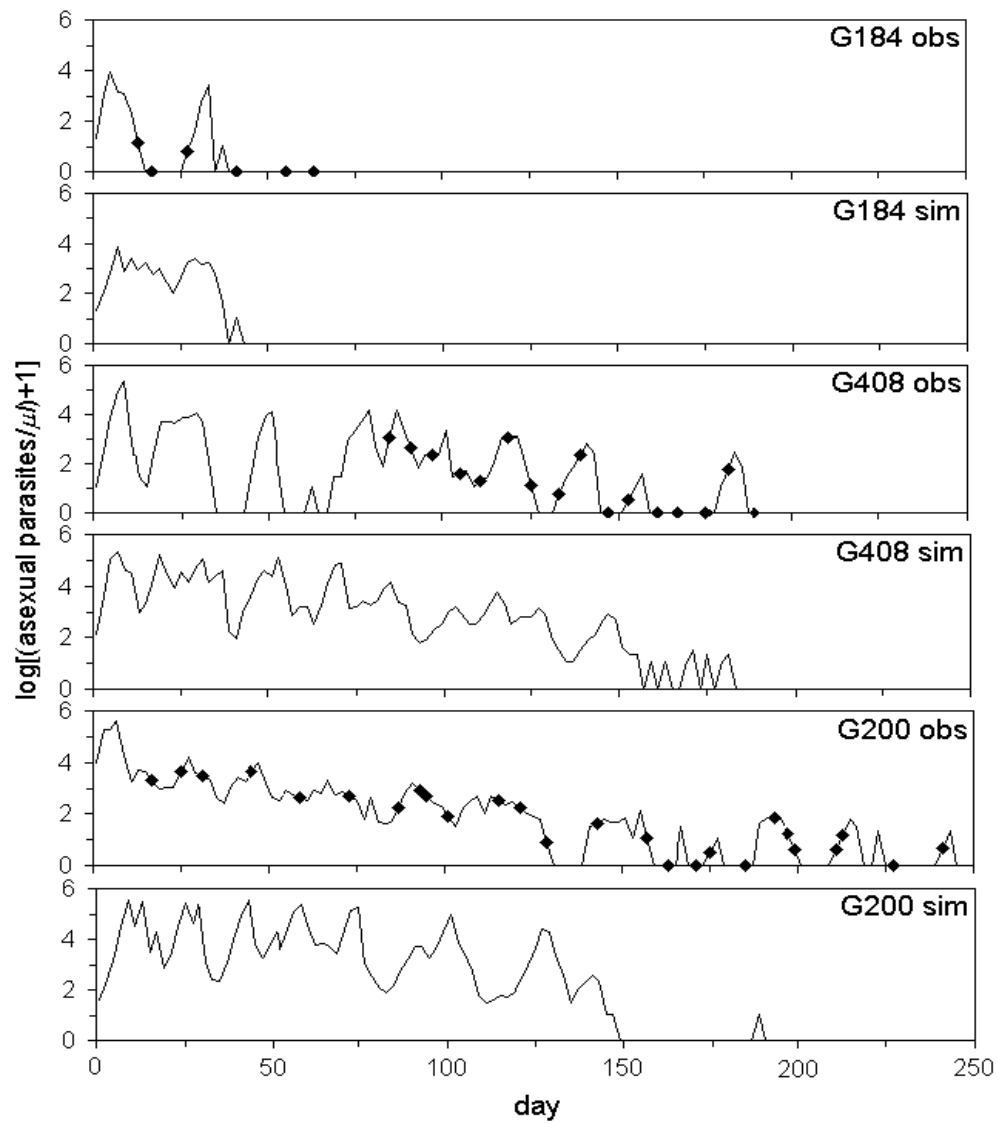


Figure 1. Three contrasting observed case-histories (odd days) and the best (by least chi-square) out of 50 stochastic realisations of the corresponding simulations.

for the acquired variant-transcending immunity is proportional to the interval from first to last day of patent parasitaemia. The simulated case histories are rather similar to the corresponding observed case histories. This was also true for the 32 remaining cases.

The relative contribution of the model's three control mechanisms to the control of asexual parasitaemia is illustrated in Figures 2 and 3.

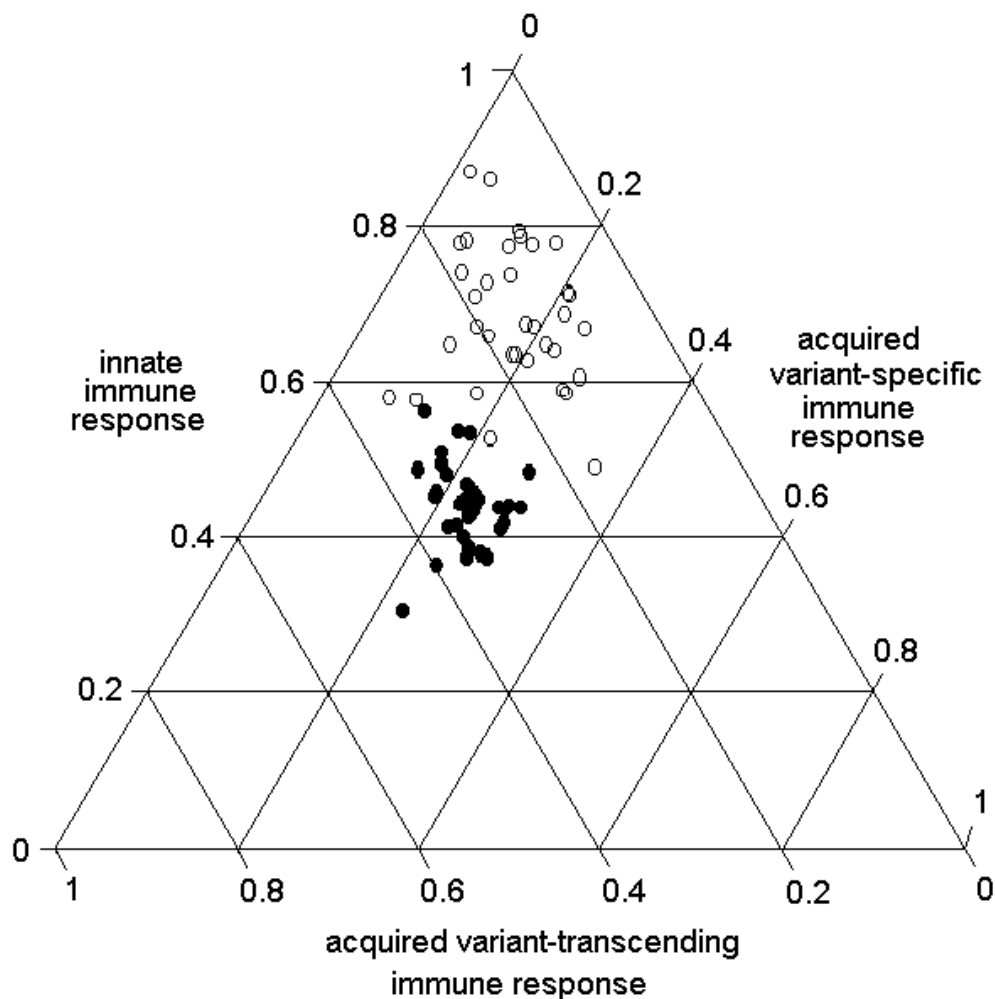


Figure 2. Ternary plot of the relative contributions of the model's three control mechanisms in the 35 simulated cases, cumulated either over the whole case-history (●) or only from onset to 6 days after the first local maximum (○).

The contributions are calculated under the following assumptions: (i) in the absence of control, the parasite population would grow exponentially; (ii) the difference between that hypothetical density and the one calculated according to the model's equations represents

the number of parasites "controlled"; (iii) the three control mechanisms compete to control the parasites; (iv) summation over a period of time gives - within that period - each controlled parasite an equal weight. Over the whole case-history, the innate variant-transcending immune response contributed 31 to 56% (median 44%) of the total control, the acquired variant-specific immune responses 13 to 29% (median 23%), and the acquired variant-transcending immune response 24 to 46% (median 33%). For the control of the early parasitaemia, up to 6 days after the first local maximum, the relative contributions of the three control mechanisms were quite different: the innate immune response contributed 49 to 87% (median 67%) of the control, the acquired variant-specific immune responses 2 to 35% (median 16%), and the acquired variant-transcending immune response 6 to 34% (median 15%).

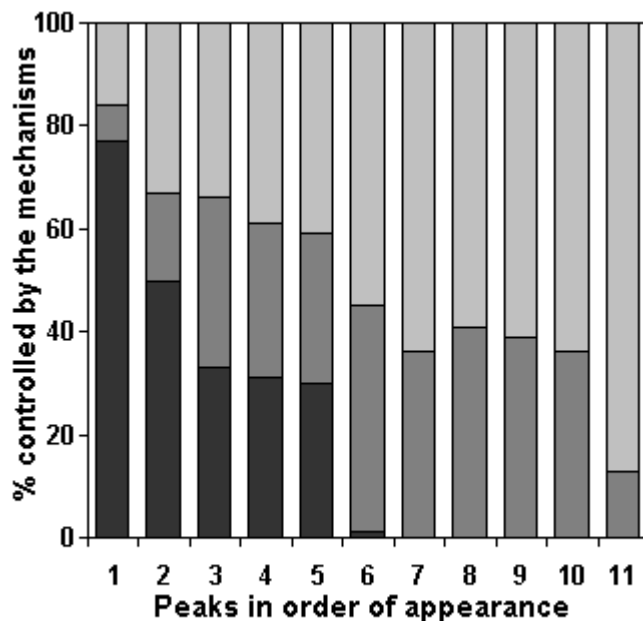


Figure 3. The relative contributions of the model's three control mechanisms (black = innate immune response; dark grey = acquired variant-specific immune responses; light grey = acquired variant-transcending immune response) in the control of the 11 successive peaks of parasitaemia of the best simulation of case G408. Each peak is identified by having a parasite density higher than the immediately three preceding values, and not lower than the three following values.

The contributions are calculated as outlined in the legend of Figure 2. According to the model, the innate immune response dominates during early control, and becomes progressively less important thereafter, while the acquired variant-transcending immune response, relatively unimportant for early control, becomes dominant in the later stages of the infection.

3.2.2 Sensitivity analysis and amendment of the model (see Publication 6)

An improved and more efficient method of fitting was developed, involving hypercube sampling and formal minimisation. The new method was used to optimise the fits and to test alternative model assumptions. The need for all five features, to which the model's realistic behaviour was attributed, was confirmed. The original model's most problematic assumption was abandoned: when offered flexible weighting of variant expression by the host's variant-specific immune status, the fitting discarded differential weighting, while producing a better fit, in particular with respect to early sub-patent periods (the original model's behaviour's most obvious shortcoming, visible in Figure 2, case G 408, see above, 3.2.1). Further improvement of the fit was obtained by applying the well-known difference between primary and secondary responses to variant-specific immunity. In terms of internal behaviour, the amended model actually expresses only a minority of the variants offered, and long infections are possible by reappearance of early variants, controlled more efficiently by a secondary response. In conclusion, the amended model simulates the course of parasitaemia much more realistically than the original, while its biological base is probably sounder. It can now be used as a tool in the discussion of potential vaccines targeting the asexual blood-stages.

3.3. A new model of gametocytaemia

3.3.1 Development and comparative evaluation of seven alternative models, and selection of the best model (see Publication 2)

(a) Abstract

In this paper, the transition of asexual blood stages of *P.f.* to gametocytes is investigated. The study is based on daily data, collected from 262 individual courses of parasitaemia. Several mathematical models that follow biological reasoning are proposed. The models are fitted with maximum likelihood and are compared with each other. The models differ in the assumptions made about the mortality of circulating gametocytes and about the transition rate of the asexual parasites. Gametocyte mortality is modelled as being (i) constant over time, (ii) linearly increasing over time, (iii) linearly increasing over gametocyte age, and (iv) exponentially increasing over gametocyte age, respectively. The transition rate is either kept constant per patient or piecewise constant within intervals that correspond to waves of asexual parasitaemia which are assumed to be caused by different Pf_{emp1} -variants. According to likelihood ratio tests, the models with age-dependent mortality rate and wave-dependent transition rates are superior to the models with constant transition rate and/or constant or time-dependent mortality rate. The best fits are

reached for models with exponentially increasing (Gompertz-type) mortality. Furthermore, an impact of high asexual parasite densities on the survival of gametocytes, interpreted as cytokine-mediated effect, is evident in some cases.

(b) *Selected results*

The fit of four models to a particular case is illustrated in the following figure.

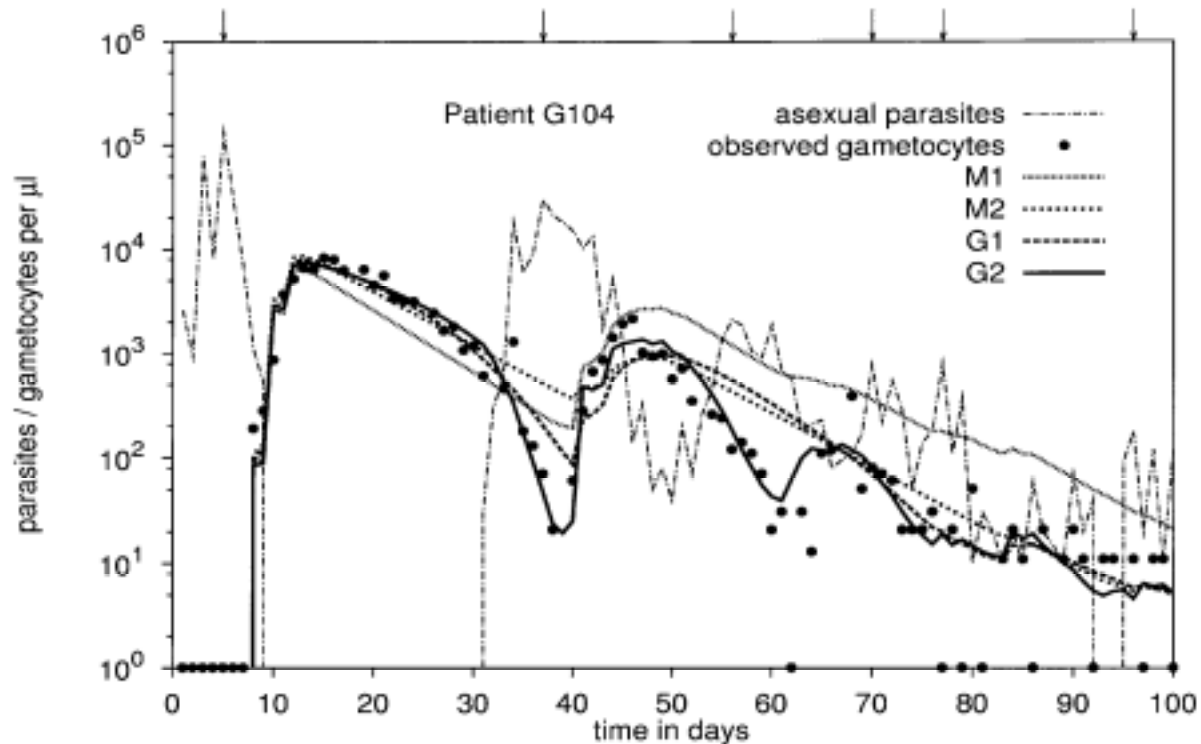


Figure 4. Comparison of the observed and expected gametocytaemia as predicted by models M_1 , M_2 , G_1 and G_2 , respectively. M_1 : constant mortality and transition rates. M_2 : constant mortality rate; this and all further models assume different transition rates for each asexual wave. G_1 : mortality rate grows exponentially with the age of the gametocytes (Gompertz-type mortality). G_2 : like G_1 , but with additional mortality (presumably cytokine-mediated) in the presence of a high asexual parasite density. The asexual parasitaemia is used as input function from which the gametocytaemia is calculated. Maxima which define asexual waves are marked with arrows at the upper margin. The four models form one branch of a hierarchical tree of seven models. The progressive gain in realism from Model M_1 to model G_2 is clearly visible. A complete compilation of all 262 individual fits is available on the internet web page (see below, 6.2)

3.3.2 *Use of the best model to estimate conversion probability and the sequestration and circulation times of P.f. gametocytes (see Publication 4)*

(a) *Abstract*

Part of the parasite's life cycle is spent in man's blood, mainly as asexual stages. A fraction of the asexual parasites develops into gametocytes (gamete precursors) while

sequestered in deep tissues. After re-entering the circulation, gametocytes can be picked up by a mosquito to continue the parasite's life cycle. The conversion probability from asexual parasites to circulating gametocytes and the gametocytes' sequestration and circulation time are estimated for the first time by fitting a dynamic model to individual patients' histories (daily records of 113 neurosyphilitic patients undergoing malariatherapy). The model assumes that the conversion probability can vary among the successive waves of sexual parasitaemia of a patient, and that gametocytes die at an age-dependent rate which increases under high asexual parasite densities. The most remarkable findings are the large individual variation of conversion probabilities and circulation times, the average gametocyte circulation time of more than twice the currently accepted value, and the large variation of conversion probability among successive waves of asexual parasitaemia without any particular time pattern. The latter finding could be explained by an association between conversion probability and variation of Pf_{EMP1} .

(b) *Remarks*

(i) The large and unordered variation in gametocytogenesis among successive waves of asexual parasitaemia cannot be explained on the basis of any of the factors advocated in the literature (mainly on the basis of *in vitro* experiments) as affecting the conversion of asexual parasites to gametocytes.

(ii) That variation should be taken into account in the thinking concerning the natural history of gametocytogenesis (including its evolution) and the control of transmission (including the potential use of vaccines, or even the genetic manipulation of gametocytogenesis).

(c) *Selected results*

The *large individual variation* of conversion probabilities and circulation times is illustrated by Table 1.

Table 1: Means and extreme values for gametocyte sequestration time, conversion probability and mature gametocyte circulation time (N=113)

Variable	Minimum	Mean*	Maximum
gametocyte sequestration time, D_s (days)	4	7.4	12
conversion probability from circulating asexual parasite to circulating mature gametocyte, \bar{g}	2.7×10^{-4}	6.4×10^{-3}	0.135
mature gametocyte circulation time, L (days)	1.3	6.4	22.2

*) "Mean" refers to the arithmetic mean for D_s and to the geometric mean for \bar{g} and L .

The large and unordered variation of conversion probability in the course of an infection is illustrated in Figure 5.

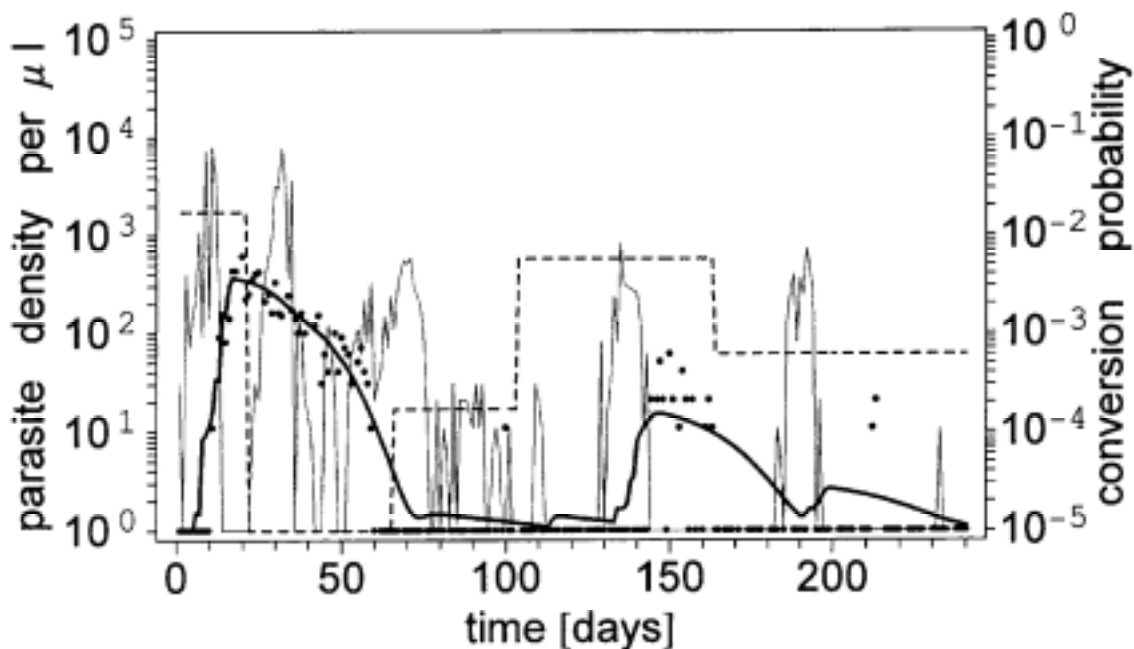


Figure 5. Comparison between model output and data in patient G221. The asexual parasitaemia (thin line) serves as input from which the parasitaemia is predicted. Parameter values are estimated by maximum likelihood to minimise the difference between observed (dots) and predicted gametocytaemia (fat line), assuming Poisson-distributed errors. The dashed step functions indicate the duration of asexual waves. The height of each step shows the estimate of the wave-dependent conversion probabilities $g(t)$ as indicated on the right hand scale.

3.4 Analysis of re-inoculation data (see Publication 5)

(a) Abstract

Malariatherapy reinoculation data were examined to detect effects attributable to individual host-specific innate factors, through correlation between descriptive variables of first and second infections. Such an effect was demonstrated with respect to the first local maximum of the asexual parasite density. The effect was seen between two *P.f.* infections, homologous or heterologous but significant only for homologous re-inoculations. The effect was also seen - and significant - between *P.f.* and *P.ovale*, but not between *P.f.* and *P.malariae*. Possible underlying factors might involve individual variation in spleen function and/or in cytokine response to malaria toxin. The data were also examined for systematic changes from first to second *P.f.* infection, as indicators of acquired immunity. The findings suggest the independent development of antiparasitic and antitoxic immunities, more straightforwardly than field studies.

(b) Remarks

While this work is analytic epidemiology, it is very relevant for modelling: (i) it supports the existence of a strong individual (presumably genetic) component of the innate immune response, as postulated in the model of asexual parasitaemia; (ii) it provides important quantitative information concerning the development and persistence of antiparasitic and antitoxic immune responses.

3.5 Work on the clinical data from Gabon

The main objective was to estimate the size and dynamics of the sequestered *P.f.* population on the basis of detailed clinical and parasitological follow-up data obtained in the course of treatment of uncomplicated malaria in partially immune children. A model was formulated and successfully fitted to the data. The data proved however insufficient to separate the estimation of two key parameters, namely (i) the fraction of sequestered parasitised RBC surviving to release merozoites, and (ii) the fraction of merozoites successfully invading uninfected RBC.

Two of the partner institutes (Kenya Medical Research Institute and Hôpital Albert Schweitzer) are engaged in collecting clinical data including additional measurements likely to allow greater discrimination in parameter estimation. Another partner (Tom Smith, Swiss Tropical Institute) is also engaged in modelling the sequestered parasite population. Continued cooperation is expected among those three institutes and the Dept. of Medical Biometry, Tübingen.

3.6 Work on the molecular (PCR-based) epidemiological data from Senegal and Tanzania

We have performed various additional analyses of the two data sets, in cooperation with the respective partners. The epidemiological interpretation of PCR-genotyping data has been extensively discussed at the last meeting. Various approaches to modelling endemic malaria including multiplicity and diversity of inoculations have been discussed at the last three meetings. Tentative simulation targets based on the Kiberege trial in Tanzania, and a tentative simulation model have been formulated. Programming of the model and its fitting to the simulation targets will, however, not be possible before the end of this Concerted Action.

4. Problems encountered

While significant progress can be claimed, less than hoped initially has been achieved for two kinds of reasons.

(a) The proposal was too optimistic. The formulation of a (successful) model of asexual parasitaemia and of a (tentative) model of molecular (PCR-based) epidemiology proved more difficult and time-consuming than expected. The malariatherapy data proved even more informative (hence again time-consuming) than anticipated.

(b) Work was delayed by events affecting two key actors: Hans Diebner (the project's part-time programmer) moved to a more attractive full-time job; Louis Molineaux (the bridge between biologists (s.l.) and modellers (s.s.)) was unavailable for several months in 1998, due to illness.

5. Technology implementation plan

Not applicable for this concerted action

6. Publications and papers

6.1 Publications

- (1) Molineaux L, Dietz K (1999) Review of intra-host models of malaria. *Parassitologia* **41**: 221-231
- (2) Diebner HH, Eichner M, Molineaux L, Collins WE, Jeffery GM, Dietz K (2000) Modelling the transition of asexual blood stages of *Plasmodium falciparum* to gametocytes. *J. Theor. Biol* **202**: 113-127
- (3) Molineaux L, Diebner HH, Eichner M, Collins WE, Jeffery GM, Dietz K (2001) *Plasmodium falciparum* parasitaemia described by a new mathematical model. (to appear in *Parasitology*)
- (4) Eichner M, Diebner HH, Molineaux L, Collins WE, Jeffery GM, Dietz K (2001) Genesis, sequestration and survival of *Plasmodium falciparum* gametocytes. Parameter estimates from fitting a model to malariatherapy data (submitted)
- (5) Molineaux L, Träuble M, Collins WE, Jeffery GM, Dietz K (2001) Demonstration of an individual host-specific factor in malariatherapy re-inoculation data (submitted)
- (6) Dietz K, Träuble M, Collins WE, Jeffery GM, Molineaux L (2001). Sensitivity analysis and amendment of a new mathematical model of *Plasmodium falciparum* parasitaemia (to be submitted after final revision)

6.2 Documents available on the Web

A complete compilation of all 262 individual gametocytaemia fits (see above, Publication (2)) is available on the internet web page

http://www.uni-tuebingen.de/biometry/eu/eu_index.html

6.3 Presentations to conferences

Selected interim results of the work on the malariatherapy data (see Publications, 6.1) were presented at conferences in Rome, Heidelberg and Accra and will be presented in Bethesda (see 2.3).

7. Conclusion

Intra-host models of *P. falciparum* asexual parasitaemia and gametocytaemia were developed that are more realistic than previous models, both with respect to their biological assumptions and with respect to their behaviour. The key elements of the approach were a critical review of the literature, the selection of appropriate primary data,

the definition of precise quantitative simulation targets, a rigorous comparison between model simulations and targets, and sustained interaction with frontline investigators in the relevant disciplines. While no model, at least in biology, should ever be considered *definitive*, the new model's realism makes them potentially useful tools to *assist* in planning and evaluation of malaria control, intervention trials, epidemiological research, research and development of new tools, especially vaccines. With respect of the new model of asexual parasitaemia, some potentially important implications concerning pathogenesis and expected effect of vaccines have been discussed at the AMVTN meeting in Accra and will be presented at the meeting "Variant proteins expressed on the surface of infected erythrocytes and their role in developing malaria vaccines", convened by the US Navy Medical Research Unit, and to be held in Bethesda on February 27-28, 2001.

In addition to results actually achieved, promising work on the estimation of the population of sequestered parasites and on molecular epidemiology of multiple and genetically diverse inoculations has been initiated. Several of the biologists invited to this meeting have been partners of the Concerted Action or were invited guests to some of its meetings.

The initial plan *aimed* at the development of better models covering the parasite's life-cycle. The project concentrated on the parasite's blood-stages, probably the part of the parasite's life-cycle that is biologically most complex and also most important for pathogenesis, and arguably the most important target of vaccine research.

**Mathematical models of the immunological and clinical epidemiology of
Plasmodium falciparum malaria**
Management Report

1. Organisation of the collaboration

1.1 Nature of the collaboration

Administratively, the project was a Concerted Action with a single contractor (University of Tübingen, Prof. Klaus Dietz). As proposed to the EC and approved by the EC, the project had two EC-funded components, interacting but distinct: (1) development and validation of *P.f.* models in the Department of Medical Biometry, Tübingen; (2) concertation among sixteen partner institutions, specialising in different relevant research areas, and wishing to participate in modelling *P.f.* malaria, either directly or indirectly.

1.2 Mode of collaboration

Collaboration was achieved through six meetings (see Sect. 2), *ad-hoc* visits, and e-mail.

1.3 Level of collaboration

A good level of collaboration was achieved, to the benefit of all participants. In particular, the modelling work in Tübingen would have been impossible without access to primary data, and benefited greatly from the partners' numerous and important intellectual inputs.

2. Meetings

2.1 Type of meeting, date, place

The following table gives type, dates and place of each of our six meetings.

No	Type	Dates	Place
1	plenary	February 4-6, 1998	Tübingen
2	plenary	November 20, 1998	Rome*
3	sub-group	September 27-28, 1999	Heidelberg **
4	sub-group	December 17-18, 1999	Tübingen
5	sub-group	May 26-27, 2000	Tübingen
6	sub-group	October 6-7, 2000	Tübingen

* satellite meeting of the Centenary Malaria Conference

** satellite meeting of the 7th Malaria EC Contract Holders Meeting

2.2 Participants

The following scientists participated in one or more of the meetings (after each name: the meetings - numbered as above - in which the scientist participated).

Members of partner teams within partner institutes

University of Tübingen

- Department of Medical Biometry: Diebner H (1-2), Dietz K (1-6), Eichner M (1-6), Molineaux L (1-6), Träuble M (4-6)

- Department of Tropical Medicine: Kremsner P (1, 3-6), Kun J (1, 4-6), Lell B (1,2, 4-6), Luty A (1, 3-6), Mordmüller B (1)

Hospital Clinic, Barcelona: Alonso P (1-2), Aponte J (1)

University of Rome: Coluzzi M (2), Costantini C (2)

Institut Pasteur, Paris

- Biomedical Parasitology: Druilhe P (1-2)

- Immunologie Moléculaire des Parasites: Arieu F (1), Puijalon O (1), Schleiermacher D (6)

London School of Hygiene and Tropical Medicine: Greenwood B (1-2)

Wellcome Trust – Kenya Medical Research Institute: Marsh (1-2)

Hôpital Albert Schweitzer, Lambaréné: Missinou M (6)

University of Oxford: Newbold C (1-2, 5)

Institut Pasteur de Dakar: Rogier C (1-2, 4-6), Spiegel A (1)

Swiss Tropical Institute: Beck HP (6), Booth M (1), Felger I (6), Smith T (1-2, 4-6)

Institut de Recherche pour le Développement, Sénégal: Trape JF (1)

University of Edinburgh: Walliker D (1-3, 6)

Guest scientists (from other institutes, or from other teams in partner institutes)

Arnot D (3-5), Day K (3,6), Deloron P (3,6), Gaillard FO (5), Hellriegel B (5), Jeffery G (1),

Kwiatkowski D (2), Pichon G (5), Theander TG (3)

2.3 Purpose and results

The purposes of the meetings (and other modes of cooperation, see above, 1.2) were to allow partners (and guests, see above, 2.1) to contribute primary data, suggestions concerning questions to be addressed through modelling, model assumptions and parameter values, constructive criticism of proposed models and their behaviour, and

orientation for subsequent work. The meetings proved very effective for all of those purposes.

3. Exchanges of personnel

There was none.

4. Problems

We wish to mention only one problem, concerning financial aspects of the contract. Because of unforeseen circumstances (see Consolidated scientific report, 4), our work was temporarily slowed down. As a consequence, our spending during the project's second year was less than expected. Following the EC's rules, our funding during the project's third year was reduced, precisely when we wanted to increase our activities. We could proceed only because the University of Tübingen was ready to lend money to its Department of Medical Biometry (within the limits of the funds originally allocated to the project by the EC), with the hope of being refunded by the EC. We hope that this report will convince the EC that the money was indeed appropriately spent, and that the EC will refund the University of Tübingen. If a similar problem were to develop between the EC and a DC contractor, the latter's institute might not be able to advance the money, so that the EC's rules may be discriminatory against DC's.

**Mathematical models of the immunological and clinical epidemiology of
Plasmodium falciparum malaria
Partners' final reports**

1. Introduction

The contributions of the partners to the modelling work in Tübingen have already been stressed in our Consolidated scientific report (see sections 2.1(d), 2.2, 2.5)

The next section (see below, 2) gives a list of recent relevant publications by partners of this Concerted Action. The final section (see below, 3) gives the reports of those partners that did actually send us an *individual* final report.

2. Relevant recent publications of the 16 partner institutions during 1997 - 2000

The papers are relevant for understanding (hence for modelling) the natural history of *P.f.* malaria in the human host, concentrating on the parasite's blood-stages. A few papers concerning other malaria species and the parasite's pre-erythrocytic stages are also included.

Each paper appears only once; the papers are listed in alphabetical order of first author; the names of partners appear in bold.

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3. Individual partner final reports (s.s.)

Activities of Dr. Christophe ROGIER (IMTSSA) as partner of the Concerted Action "Mathematical Models of the Immunological and Clinical epidemiology of *Plasmodium falciparum* Malaria

As the chief of the malaria epidemiology laboratory of Institut Pasteur of Dakar from October 1989 to August 1996, Dr C. Rogier had in charge the daily follow up of the population of two villages in Senegal, Dielmo (250-300 inhabitants, holoendemic malaria) and Ndiop (300-350 inhabitants, mesoendemic malaria). His contribution to the Concerted Action included expertise in malaria epidemiology, experience in statistical modelling of the relationship between transmission, parasitemia, (density and complexity), disease and immunologic response, and providing different sets of data from these longitudinal studies to the Institut für Medizinische Biometrie (Prof. Dr. Klaus DIETZ), Tübingen.

The studies were carried out in collaboration with JF Trape (IRD), O. Puijalon & P. Druilhe (Institut Pasteur, Paris). The main lessons from these longitudinal studies follow. They were worth for modelling the immunological and clinical epidemiology of *P. falciparum* malaria.

Study of the relationships between malaria transmission, infection and morbidity

Measuring malaria morbidity

The populations of Dielmo and Ndiop have been enrolled in a longitudinal study of malaria, respectively since 1990 and 1993. Similar entomological surveys and identical strict clinical surveillance programs are carried out, including a daily home visit to each person and the presence, night and day, of a medical team in the villages. Any pathological episodes are diagnosed and treated. The perception of the symptoms, the medical vocabulary, the knowledge, the attitudes and the practices related to feverish diseases were almost the same in the two populations. Malaria transmission intensity differs considerably in these two villages that are only 5 kilometres apart. In Dielmo, transmission is intense, about 200 infective bites per person per year, and perennial (with seasonal fluctuations) due to the presence of a stream which serves as a permanent breeding site. In Ndiop transmission is about eight to ten times lower and only occurs during the four months of the rainy season.

Investigating longitudinally the intrinsic nature of the relationship between parasitaemia and fever at the individual level and the variations in tolerance of parasitaemia among individuals in the population of Dielmo, the evidence for an age-dependent threshold effect of the parasitaemia was obtained. The level of this threshold varied by 2.45 trophozoites per leukocyte, maximum at one year of age, to 0.5 trophozoites per leukocyte, minimum after 60 years of age. When the parasite density of a person crossed the threshold level corresponding to his or her age, the individual's risk of fever was sharply increased. In Ndiop, malaria attacks are also associated with sharp increase in parasite density but the level of parasitaemia triggering fever is lower and its variations according to age are less pronounced.

From 689 *P. falciparum* malaria attacks observed and treated during a three year period among 226 inhabitants (78% of cases < 6 years old) of Dielmo, neither convulsion nor life-threatening anaemia were recorded and only one case was severe and died. The symptom frequencies observed during these 689 malaria attacks were tested against age, gender and parasite density and a research of distinguishable clinical presentations was carried out by multi-correspondence analysis. There was little difference between the severity of symptoms during the initial course of attacks in young children and adults, and this severity was not correlated with the duration of the pathological episode. It was not possible to distinguish objectively different malaria attack types according to the severity of clinical manifestations. In contrast, the duration of fever, symptoms, and parasite clearance were significantly longer among the youngest children than among the oldest children and adults. These findings suggest that of the two components of protective immunity - anti-parasite and anti-toxic immunity - only the first would play a major role as age increases. They suggest also that the initial clinical presentation of malaria attacks is not predictive of the level of protective immunity.

These published observations are worth for modelling the relationship between parasitemia, immunity and clinical manifestations of malaria.

The level of transmission as a risk factor for malaria morbidity

The malaria morbidity in Dielmo and in Ndiop, under different transmission level conditions, has been compared. The patterns of age-dependent variations in mild malaria attack incidence differ markedly in the two populations. The proportion of attacks arising during adulthood was respectively 23% and 41% in Dielmo and in Ndiop. According to these observations, at the age of 60, the inhabitants experience an average of 43 and 62 malaria attacks in Dielmo and in Ndiop respectively, whereas they are respectively

exposed to about 200 and 20 infective bites per person and per year. The comparison of these populations indicates that the burden of malaria morbidity is similar in populations that vary by as much as a factor of 10 in exposition to transmission.

In most of African tropical areas, the level of malaria transmission varies according to the seasons and the rainfalls. The fluctuations of the entomological inoculation rate and the incidence density of malaria attacks in Dielmo and in Ndiop are closely related, and a tenfold decrease or increase in malaria transmission is associated, in the following weeks, with a twofold decrease or increase in malaria morbidity.

The relationship between the susceptibility to malaria i.e. the risk of clinical malaria attacks or the risk of patent parasitemia per infective bites, and the intensity of transmission follows an inverse function with a maximum risk when the intensity of transmission is low and a minimum risk reached with higher levels of transmission. The same pattern of relationship is observed whatever the age groups, from children aged less than 1 year up to adults whose risk is lower. The adjustment of this clinical resistance to the level of transmission seems to be rapid (some days to some weeks). These observations suggest that one or several mechanisms control parasitaemia and occurrence of clinical attacks in a density-dependent manner. These mechanisms may be related to a rapidly acquired and short lived density-dependent immune response (against pre-erythrocytic stage ?) or/and to parasite competition (including an hypothetical active inhibition of parasite populations by others or a competition between parasites for receptors, mediators or nutriments). These published and unpublished observations are worth for modelling the relationship between transmission, immunity and clinical malaria.

Incidence, risk and predictive factors of clinical malaria

The observations made during short periods in Dielmo suggest that the acquisition of clinical protection in areas where malaria is highly endemic involves a progressive and homogeneous decrease of the probability of having a malaria attack (attacks occur less frequently as age increases, in all children), rather than the acquisition of complete protection by an increasing number of children. However longitudinal data collected for several years from Dielmo and Ndiop show important differences between children in acquiring anti-parasite and anti-disease immunity. Some children had only one malaria clinical episode before the age of two years while others experienced 20 times more malaria attacks during the same period. These differences were undetectable through studies lasting only a few months and were not explainable by the phenotype of haemoglobin, the G6PD activity level, the HLA groups, the bed nets use or the location in

the village. The evidence of the clinical protective effect of cytophilic antibodies (mainly IgG3) against *P.f.* offer an example of immunological mechanism involved in immunity and the opportunity of estimating its role.

It is well known that women are more susceptible to malaria during pregnancy. Analysing 71 histories of women from Dielmo who were monitored daily during the 33 month-period which covered twelve months before conception, the whole duration of pregnancy, and twelve months after delivery, the incidence rate of *P.f.* malaria attacks was, on average, 5.5 (95% CI: 2.6-11.7) times higher during the second and third trimesters of pregnancy than during the year preceding conception. This increase in risk of clinical malaria was greater among primigravidae than multigravidae but was detectable up to the fifth pregnancy. The possible persistence of this phenomenon during postpartum was also investigated. High incidence of malaria attacks persisted three months after delivery. During this period, the incidence rate of malaria attacks was, on average, 3.6 (1.7-7.7) times higher than observed during the year which preceded pregnancy and during the 4th to 12th months after delivery. Parasite prevalence and mean asymptomatic parasitaemia were also increased during pregnancy and early postpartum compared to other periods. These findings indicate that women are highly susceptible to malaria both during pregnancy and early postpartum, and support the hypothesis that pregnancy-associated immuno-suppression, but not only parasite sequestration in the placenta, is the leading mechanism involved in maternal malaria.

Genotyping P. falciparum

Parasite typing was carried out using a PCR-based molecular analysis of allelic polymorphism. The parasite populations circulating in the villages of Dielmo and Ndiop are characterized by a considerable allelic diversity of the MSP-1, MSP-2 and TRAP loci. There was geographical variations in the distribution of alleles or of allelic families between the two villages (only 5 km apart). A longitudinal survey of the circulating genotypes has been carried out over a one year period in Ndiop. Comparing two surveys conducted one month apart during the rainy season, i.e. the season where parasites are actively transmitted from one person to the next, the allelic distribution was similar. Comparing surveys over the dry season (where the transmission is undetected), the parasite population were very different. This was due to substantial variations occurring during persistent chronic carriage in the dry season.

A large proportion of blood samples contained several MSP-1 or MSP-2 alleles. In asymptomatic carriers, the complexity of the infections (number of alleles) was age-

dependent in Dielmo and was age-independent in Ndiop. The complexity is increased during pregnancy. In children, the trend was for a reduced complexity during clinical episodes. Molecular typing showed that i) successive clinical episodes experienced by children were caused by genetically distinct parasites, ii) clinical episodes are associated with rapid multiplication of recently inoculated parasites which reach high density (above a pyrogenic threshold), and iii) children control multiplication of some genotypes and then the infections remain asymptomatic. Longitudinal analysis of asymptomatic carriers indicated that in absence of transmission, the same parasite types were carried for long periods, while rapidly changing profiles were observed during intense transmission season. These observations are worth for intra host modelling of the *P. falciparum* infection.

Providing sets of data

Several sets of data were provided to the Institut für Medizinische Biometrie (Prof. Dr. Klaus DIETZ), e.g. longitudinal clinical and parasitological observations from inhabitants of Dielmo or genotypes of *P. falciparum* populations observed longitudinally among pregnant women.

Activities of Dr T Smith (Swiss Tropical Institute) during the course of the Concerted Action.

As head of the Biometrics unit of the Swiss Tropical Institute, Dr Smith has been engaged in a number of research projects relevant to the Concerted Action. While the Concerted Action did not directly fund these projects, the meetings of the partners were an important contribution to the development of the scientific thinking of these areas of work.

Dynamics of malaria infection in endemic areas

Until recently, dependence on light microscopy for assessment of malaria parasitaemia in the field has precluded estimation of either the force of infection or the duration of individual malaria infections in areas of high transmission. This is because it is impossible to distinguish new infections morphologically from persisting infections in longitudinal studies. As a result, mathematical models of malaria transmission have been based on very indirect estimates of the force of infection and of infection duration. Trials assessing effects of interventions on infection rates have only been possible if a radical cure of parasitaemia was carried out at the start of the study.

PCR techniques for detecting parasites have made it possible to identify parasite clones longitudinally, but do not necessarily detect all the clones in a host, since some parasite clones may be completely sequestered, densities may be beneath the detection limit (or PCR artefacts may prevent amplification). However, longitudinal analyses can be used to estimate the numbers of sub-patent genotypes from transition rates using a high-resolution system, such as PCR-RFLP analysis of *m*sp-2. The Swiss Tropical Institute partners are developing statistical models based on Hidden Markov Models, to simultaneously estimate the detectability of individual parasite clones, the force of infection for each genotype and the average duration of infection.

A recent trial of impregnated bednets in the village of Kiberege in Tanzania was analysed using these methods. It was found that over the age range from 6 months of age to 30 months, there was a considerable increase in the average duration of malaria infections, but no age-dependence in the infection rate. This result calls into question immunological models that assume that the acquisition of immunity will necessarily result in infections being cleared more quickly. A field study currently in progress in Navrongo, Ghana, is testing to what extent this result can be generalised to other settings, or indeed, whether the age trends continue into older age groups

Estimation of the sequestered load

Plasmodium falciparum undergoes an obligate stage of cytoadherence to endothelia, during which parasites are undetectable by microscopic examinations of peripheral blood. Despite the importance of sequestered parasites in pathogenesis, there is no practicable or generally accepted technique for quantifying the overall degree of synchronisation of the parasites or the load of sequestered parasites *in vivo*. This is a major limitation in diagnosis, and a methodological problem for pathophysiological and epidemiological studies. We aim to overcome this limitation by developing both biochemical and statistical approaches to estimate sequestered loads. Currently, we are developing statistical models to estimate the load of sequestered parasites from longitudinal studies of parasite dynamics in severe malaria patients. A Markov Chain Monte Carlo approach is currently being fitted to data from patients in Kilifi Hospital, Kenya. The next stage will be to study the dynamics of host and/or parasite products released during schizogony and schizont rupture, and to identify candidate biochemical markers of the extent of sequestration. These will then be cross-validated against the statistical models, in order to provide a method for routine assessment of the sequestered load of malaria parasites.

Multiple infections of Plasmodium falciparum

A series of studies on the epidemiology of multiple *P. falciparum* infections were reported in a supplement to the *Transactions of the Royal Society of Tropical Medicine* in 1999. This research was centred on the results of PCR-RFLP typing of the *msp2* locus of *P. falciparum*. Most of the work was carried out in the Kilombero valley of Tanzania, where children between 3-7 years of age were found to harbour on average 5 genotypes of parasites at once. Studies included both cross-sectional and longitudinal analyses of multiple *P. falciparum* infection in infants, and in older children up to 30 months of age. We also carried out comparisons of multiplicity in sick children with that in healthy controls, and cross-sectional surveys of multiplicity over the whole age range of inhabitants of the Kilombero valley.

The studies reported in the supplement were carried out during the period of the previous report, where they are described in detail. We continue to explore the consequences of our findings, especially the importance of measurements of multiplicity of infection in malaria epidemiology. Our findings led us to reassess the nature of semi-immunity in malaria and have relevance not only for vaccine development and testing, but also for our understanding of malaria ecology and the evolution of virulence.

One important conclusion of these studies was that asymptomatic infections with *P. falciparum* can protect against clinical episodes caused by superinfecting parasites. This idea is a key element in a model proposed for the way clinical immunity to *P. falciparum* develops in highly endemic areas. Others have also suggested that parasites belonging to other species (in particular, *P. malariae*) might protect against clinical *P. falciparum* attacks. We set out to assess these hypotheses using three approaches: (1) simulation modelling of the development of clinical immunity, to assess the consistency of our model with data from Tanzania; (2) analysis of databases from the Wosera area of Papua New Guinea to describe the epidemiology of different species of malaria there, and how they interact; (3) further field studies in São Tomé to determine whether the results can be generalised to areas of different endemicity.

Simulation model of multiple infection in P. falciparum

The stochastic simulation model of natural immunity was programmed with a user-friendly interface, which enables the user to experiment with different values for the parameters of the protective responses. Validation of this simulation model against the observed distributions of the target variables from the field is in progress.

Analysis of existing data from Papua New Guinea

The analysis of the Papua New Guinea database considered longitudinal patterns of infection and morbidity, based on a database of 10,128 malaria slides examined during 1990-92, together with entomological exposure data collected at the same time. The first stage involved understanding the effects of age and entomological exposure on *P. vivax* and *P. malariae* in this population. Average entomological inoculation rates estimated from indoor landing rates on individuals without bednets were 35, 12 and 10 infectious bites per person per annum for *P. falciparum*, *P. vivax* and *P. malariae* respectively.

However, the protracted use of bednets appeared to considerably modify malaria endemicity, even where coverage was incomplete and without insecticide treatment, with age-prevalence patterns for *P. falciparum* varying between typical patterns for meso-endemic and holo-endemic areas. This appeared to be because long-term use of untreated bednets reduces sporozoite rates. Even when there was a high bednet coverage most Anophelines fed on human hosts, so the decreased sporozoite rates are likely to be largely due to reduction of mosquito survival. These results highlight the importance of local vector ecology for the outcome of bednet programmes, and suggest that area effects of untreated bednets should be reassessed in other settings.

The analyses of interactions between the three malaria species found that *P. malariae* infection was positively associated with homologous infection four months previously and with prior *P. falciparum*, but not *P. vivax* infection. Prospective analysis of health-centre morbidity supported the idea that *P. malariae* protects against disease, but indicated greater protection against non-malaria fevers than fever associated with *P. falciparum*. *P. vivax* appeared to protect against *P. falciparum* disease but not against other morbidity. Confounding variables could account for many differences among reports of inter-species interactions in human malaria.

Field Studies in São Tomé and Príncipe

A field survey of 491 individuals was carried out in the Riboque area of São Tomé town. PCR-RFLP analysis of the *msh-2* locus of *P. falciparum* was carried out in order to test whether multiple infections protect against subsequent morbidity. All attendances with fever at a health post within Riboque were recorded during the following year. The results of the PCR-RFLP typing and the first three months of follow-up confirmed the protective effects of multiple infections, but raised issues regarding the specificity of the protection.

A further survey including over 1000 people carried out on the nearby island of Príncipe, coordinated with a PCD system at the island hospital, provided potentially valuable baseline data for control activities and demonstrated relationships between malaria infection and bednet use and house construction type. However, *P. malariae* and *P. vivax* were both less frequent than in previous surveys on the island. Preliminary results suggest that, in contrast to the situation in Papua New Guinea, in São Tomé and Príncipe *P. malariae* offers no protection against subsequent *P. falciparum* morbidity.

**Meeting of EC Concerted Action Project
on Modelling of *Plasmodium falciparum* Malaria**

Tübingen, December 17 and 18, 1999

Agenda

1. Formulation of the model:
 - variables and parameters
 - shape of relationships
 - outputs comparable to data

3. Data sets available for validation (covering the range from low, unstable and seasonal to high, stable and perennial transmission)
 - Senegal (Dielmo, Ndiop)
 - Tanzania
 - Sudan
 - others?

4. Plan of work for the third (and last) year of the project

Participants: Arnot D, Dietz K, Eichner M, Kremsner P, Kun J, Lell B, Luty A, Molineaux L, Rogier C, Smith T, Träuble M

**Meeting of EC Concerted Action Project
on Modelling of *Plasmodium falciparum* Malaria**

Tübingen, May 26 and 27, 2000

Agenda

1. Latest results of work based on the malariatherapy data
 - 1.1 Comparison – in terms of assumptions and outputs – between two ways of modelling variant expression, respectively based on immune regulation of expression itself, or on partial cross-immunity of each variant with a subset of the others
 - 1.2 Correlation between the characteristics of a first malaria inoculation (*with P. falciparum, P. malariae or P. ovale*) and those of a second malaria inoculation (*with P. falciparum*)
2. Development of the model of clonal diversity and polyclonal infections
 - 2.1 Discussion of simulation targets
 - 2.2 Discussion on the design of a model that would be likely to meet the simulation targets.
3. Plan of action or the remainder of the EC Contract, including a possible further meeting
4. Possibility of continued cooperation, beyond the end of the EC-Contract (November 30, 2000)

Participants: Arnot D, Dietz K, Eichner M, Gaillard D, Hellriegel B, Kremsner P, Kun J, Lell B, Luty A, Molineaux L., Newbold C, Pichon G, Rogier C, Smith T, Träuble M

**Meeting of EC Concerted Action Project
on Modelling of *Plasmodium falciparum* Malaria**

Tübingen, October, 6 and 7, 2000

Agenda

1. Presentation and discussion of work accomplished or in progress
 - 1.1 Sensitivity analysis of the model of *P.f.* asexual parasitaemia, including:
 - (a) the substitution of immunoregulation of variant expression by restricted and partial cross-immunity among variants;
 - (b) simulation of expected effects of some hypothetical new tools.
 - 1.2 Interpretation of epidemiological studies using PCR genotyping of *P.f.*
 - 1.2.1 What epidemiologists, statisticians, modellers should know about PCR genotyping techniques
 - 1.2.2 Two statistical problems
 - (a) Estimating “true multiplicity of infection” from PCR genotyping data
 - (b) Comparing the *P.f.* allele frequency distributions in different human populations
 - 1.2.3 Epidemiological inferences from field studies using PCR genotyping
 - (a) Presentations by the five laboratories represented at the meeting (Basel, Edinburgh, Oxford, Paris, Tübingen)
 - (b) Testing randomness of recombination between MSA 1 and MSA 2 alleles in the data of Schleiermacher et al.
 - 1.2.4 General discussion
 - 1.3 Formulation of a cohort model of *P.f.* asexual blood stage infection, allowing multiple introduction of diverse clones, and testing of such a model against field data, including parasitaemia, PCR genotyping and incidence of malarial fever
2. Preparation of the final report of the current EU project: contributions expected from the partners
3. Further co-operation: do we want to continue? If so, how?

Participants: Beck HP, Day K, Deleron P, Dietz K, Eichner M, Felger I, Kremsner P, Kun J, Luty A, Missinou M, Molineaux L, Rogier C, Schleiermacher D, Smith T, Träuble M, Walliker D

**Mathematical models of the immunological and clinical epidemiology of
Plasmodium falciparum malaria**

Completed catalogue page

Results achieved

The *approach* described in the summary was followed very closely. A critical review of published within-host models of malaria, and the development and validation of two new models, respectively of the asexual parasitaemia and the gametocytaemia of *P. falciparum* within the human host was completed. In comparison with previous models, the new models take better account of current biological knowledge, and produce more realistic simulations. They are potentially useful tools for helping to plan research and development concerning *P. falciparum* malaria and its control. The model of asexual parasitaemia, in particular, through distinguishing among different within-host parasite control mechanisms, has important implications for *P. falciparum* induced pathogenesis and its control.

Data sheet for final report

1. Dissemination activities

Published Submitted

Number of communications in conferences	1	
Number of communications in other media (internet, video, ...)		
Number of publications in refereed journals	3	3
Number of articles/books		
Number of other publications		

2. Training

Number of PhDs	
Number of MScs	
Number of visiting scientists	
Number of exchanges of scientists (stay longer than 3 months)	

3. Achieved results

Number of patent applications	
Number of patents granted	
Number of companies created	
Number of new prototypes/products developed	
Number of new tests/methods developed	
Number of new norms/standards developed	
Number of new softwares/codes developed	2
Number of production processes	
Number of new services	
Number of licenses issued	

4. Industrial aspects

Industrial contacts	yes	<input type="checkbox"/>	no	<input checked="" type="checkbox"/>
Financial contribution by industry	yes	<input type="checkbox"/>	no	<input checked="" type="checkbox"/>
Industrial partners : - Large	yes	<input type="checkbox"/>	no	<input checked="" type="checkbox"/>
- SME ⁶	yes	<input type="checkbox"/>	no	<input checked="" type="checkbox"/>

5. Comments : Other achievements (use separate page if necessary)

⁶ Less than 500 employees