12

Evolution of parasite virulence to vectors

S.L. Elliot [1, 2], Maurice W. Sabelis [1] and Frederick R. Adler [3]

Abstract

Vectorborne parasites are commonly predicted to be less virulent to the vector than to the definitive host as the parasite gains little by harming its main route of transmission. Here we assess the empirical evidence from systems where insects vector vertebrate parasites. The body of evidence supports lower (but non-zero) parasite virulence to vectors than to plant or invertebrate hosts but not to vertebrate hosts. We consider why this might be by assessing evolutionarily stable strategies for an insect parasite that can infect both vector and definitive host and can have distinct virulences in these two potential hosts. In a homogeneous environment the parasite is predicted to be equally virulent to vector and host. However, in a patchy environment it is predicted to become benign towards the more mobile of the two potential hosts, provided competitive displacement among strains in a patch is weak. This prediction may not meet reality for two different reasons. First, relative mobility of vector to host depends on the spatial scale under consideration: malaria mosquitoes are the more mobile hosts from house to house within a human settlement, but human hosts may be more mobile from one settlement to the other. Second, as in malaria, the main host and therefore probably also the vector may be multiply infected and this is likely to increase virulence and to level off differences between vector and definitive host.

Keywords: Evolution of virulence; vectorborne disease; dispersal; superinfection; free parasite

Introduction

Vectorborne parasites and pathogens are among the most damaging of disease-causing organisms, be they of medical, veterinary or agricultural importance (Ewald 1994; Power 1992; Dieckmann et al. 2002). Management of these diseases has traditionally been from a population dynamical stance, principally directed at controlling populations of the vectors or enhancing the resistance of the hosts. The likely impact of such interventions upon the evolution of parasite virulence has recently received theoretical attention (Gandon et al. 2001), and is part of a growing field which seeks to manage the virulence of disease-causing organisms (Dieckmann et al. 2002). Despite this interest, the only general predictions which have been made

[1] Section Population Biology, Institute for Biodiversity and Ecosystem Dynamics, Universiteit van Amsterdam, Postbus 94084, 1090 GB Amsterdam, The Netherlands. E-mail: sabelis@science.uva.nl
[2] NERC Centre for Population Biology, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, UK. E-mail: s.elliot@ic.ac.uk (Author for correspondence)
[3] Department of Mathematics and Department of Biology, 155 South 1400 East Salt Lake City, UT 84112-0900, USA. E-mail: adler@math.utah.edu

for vectorborne diseases are (1) that they will be more virulent than non-vectorborne parasites, and (2) that parasites will have a lower virulence to their vectors than to their main hosts (Ewald 1994). Whatever the validity of these generalizations, they rest upon some critical assumptions, have yet to be updated in the light of a body of theoretical work on the evolution of virulence over the last decade or so (but see also Day 2001; 2002), and have not been considered in terms of biological differences between systems which may affect predictions. Our aim is to question some of these assumptions and provide a framework within which modern theory can be applied so as to generate testable hypotheses.

Critical to our approach is the recognition that many parasites reproduce in the vector as well as in the main host and that this may harm not only the main host but also the vector. There will thus be selection upon the parasite's virulence toward the vector just as there is toward the host. Indeed, definitions of the 'vector' and its converse, the 'main' or 'definitive' host, serve to ascribe functions to what are, in effect, two potential hosts on different trophic levels (i.e. where one feeds upon the other, perhaps as a 'micropredator'). A parasite can therefore have two distinct virulences to these two hosts and our contention is that these virulences are so intimately related that consideration of one requires consideration of both. These two virulences will be subject to natural selection due to a range of factors, for example spatial heterogeneity of hosts, their mobility and life histories, or competition between parasite strains. A further justification for our approach is that survival of the vector and its ability to transmit the parasite are key factors in the dynamics of vectorborne diseases, both liable to be influenced heavily by harmful effects of the parasite.

We consider vectorborne diseases of vertebrates and, in particular, humans. Common to all is that it is an arthropod which serves as the vector.
We begin with a general ESS model of parasite-vector-host systems. In this model we investigate the roles of spatial heterogeneity, host mobility and superinfection (the ability of strains to replace one another in a patch) in virulence to vector and main host. We then consider how the different biological features of these systems will influence selection on virulence, in the light of the model and current theory. We identify what patterns are already apparent from the literature and to highlight hypotheses which seem to merit particular attention. Our intention is to emphasize particular questions which need addressing empirically and theoretically.

Patchiness, mobility and virulence

In his book *Evolution of Infectious Diseases*, Ewald (1994, p. 47) gave an explanation for the apparently lower virulence of parasites in vectors than in main hosts: "…vectorborne parasites should specialize on their vertebrate hosts as resource bases for amplifying their numbers and on their vector hosts as agents of dispersal". This makes good intuitive sense. However, to subvert it somewhat, the parasite is just as reliant upon the main host for transmission to new vectors as the reverse. So, which potential host is more important to the parasite? At the heart of this and other predictions is that the vector represents mobility to the parasite. This therefore serves as the first biological feature to consider – how does the mobility of *either* host affect the ESS virulences of the parasite? This requires a consideration of the patchiness of the hosts so we include this in our ESS analysis. We assume no cost to dispersal between patches, exclusive transmission via vectors, and a special form of multiple infection, termed superinfection (Dieckmann et al. 2002). We do allow dispersal by

movement of infected hosts. The model presented in Appendix 1 is based on a paper by Elliot, Adler and Sabelis (submitted).

What we find is that in an unstructured population of potential hosts, the ESS virulence to predator and prey is equal (see Appendix 1). Referring to the quotation above, we can in this case state that the parasite relies as much on the host for transmission to the vector as the converse. Once we introduce spatial heterogeneity in the form of multiple patches, each containing predator and prey, we find that ES virulence is lowest in the vector. This is to be expected as the vector must live long enough to leave the patch and reach a new one. However, this intuitively reasonable result is sensitive to the intensity of within-host parasite competition (see Figure 1). If we increase the rate at which co-occurring parasite strains can outcompete one another, virulences also converge, but to a limited degree when only vectors disperse pathogen between patches. This is because the parasite must exploit the patch before losing it to the competitor. So only in a patchy environment, with little competition between parasite genotypes and a dependence on live hosts for transmission between patches, can we expect a lower virulence to the more mobile host. Why, then, are vectors generally considered to be little affected by the parasites they bear? Are the conditions set out in this ESS analysis common features of parasite-vector-host systems, or must we look for other differences between the potential hosts to explain differences in virulence? Is there actually any empirical evidence of lower parasite virulence in vectors, or is there a bias in the diseases that are studied or in how they are studied? To address these questions, we now consider vectorborne disease of vertebrate hosts.

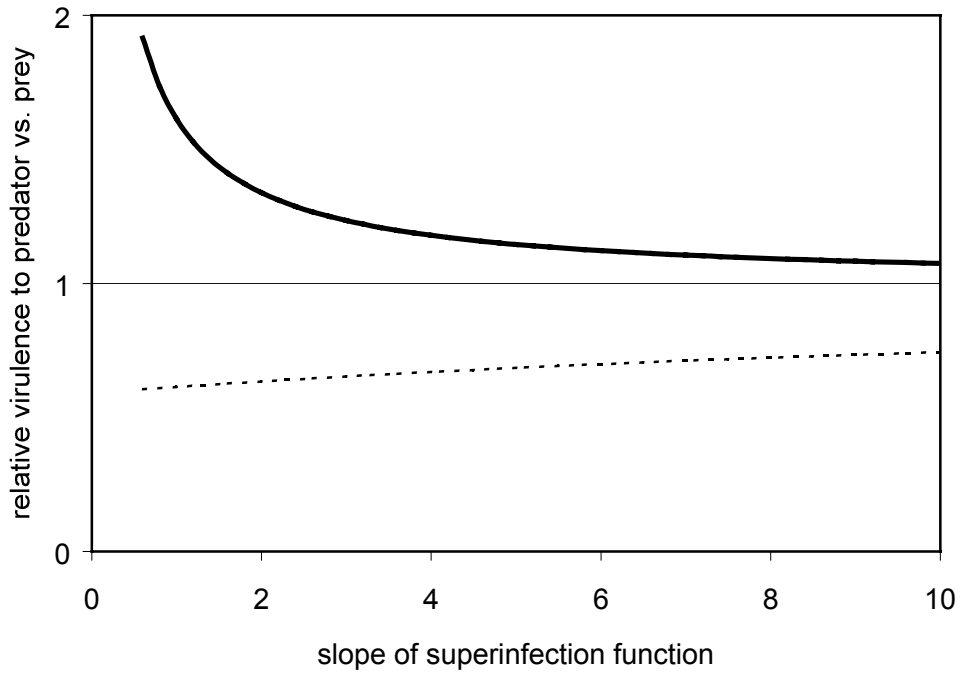

Figure 1. Model predictions of relative ESS virulence of a parasite to mosquitoes versus hosts in relation to the slope of the superinfection function. Bold lines, hosts only move between patches; dashed lines, mosquitoes only move between patches.

Vectorborne diseases of vertebrates

It is for vertebrate diseases that parasite effects on vectors have been most studied. As with many plant parasites, we would expect an effect on the vector as the parasite must pass through host tissues and use host resources for multiplication (Randolph 1998; Welburn and Maudlin 1999; Ghosh, Edwards and Jacobs-Lorena 2000; Kollien and Schaub 2000), both of which are liable to cause some harm to the vector (Mims, Day and Marshall 1966; Lam and Marshall 1968; Maier, Becker-Feldman and Seitz 1987; Beier 1998; Zieler and Dvorak 2000; Ferguson and Read 2002). Such effects could partly explain the development of resistance ("refractoriness" or "incompetence") in vectors (Welburn and Maudlin 1999; Yan, Severson and Christensen 1997). Arboviruses can negatively affect the development time, survivorship or lifespan of their mosquito vectors (Turell, Gargan and Bailey 1985; Faran et al. 1987). While the evidence from malarial parasites in mosquitoes has been ambiguous (Chege and Beier 1990) and the experiments conducted have been criticized for use of unrealistically high infection rates and a lack of field corroboration (Chege and Beier 1990; Taylor and Read 1997), the overall pattern is of malarial parasites reducing mosquito survival (Ferguson and Read 2002). Even though a bias towards diseases of particular virulence to vertebrates may be expected, case-fatality rates in vertebrates can be as low as 1% (e.g. Snow et al. (1999)). No concerted effort has been made to compare virulences in a vector and a vertebrate host and experiments are clearly complicated by practical and ethical issues. However, some degree of virulence has been shown in vectors, sometimes in subtle forms such as reductions in fecundity, as with mosquitoes and blackfly (Turell, Gargan and Bailey 1985; Hurd, Hogg and Renshaw 1995). These effects can be seen as an adaptation of the parasite as fecundity effects would not hamper parasite transmission (unlike plant parasites whose vectors multiply on the plant host before dispersing and vectoring parasites). We must therefore question whether predictions of lower virulence to vectors of vertebrate diseases have any empirical basis, and explore why this may not be the case.

Recipes for challenging predictions

We have sought conditions in which predictions of lower virulence toward vectors than "main" hosts will hold, but the ESS model predicts it to be highly subject to specific conditions of spatial heterogeneity, differential mobility of the two hosts and weak competition between parasite genotypes. For vectored diseases of vertebrates there is a critical issue related to the spatial scale of mobility: what are patches and how mobile are the hosts between them? If patches are human habitations then we may expect greater vector mobility between these than human mobility. However, if patches are human villages or towns, then human mobility between these will almost certainly be greater than vector mobility. We may therefore expect selection on virulence to act in opposing directions at the two spatial scales.

Another critical issue is the intensity of within-host competition. For example, there is strong evidence for multiplicity of *Plasmodium* genotypes and species in individual human hosts in holoendemic areas (e.g. Smith et al. 1999; Bruce et al. 2000). This may well explain why malaria parasites are not mild to their definitive host. But what do we know of parasite competition in the mosquito vector? One may argue for a lower probability of multiple infection in the vector than in the main host. An individual mosquito takes a limited number of blood meals per day and each blood

meal represents an extremely small fraction of the total blood mass of the victim. Thus, the diversity of malaria parasites entering the gut of the mosquito are likely to be much smaller than present in the blood of a human host. However, lower parasite diversity does not necessarily mean less competition among parasites. In one or another generation of vectors, a given parasite genotype will face within-vector competition with its superior competitor genotype. The critical issue is whether there is a sufficiently high probability for vectors to be infected by a single *Plasmodium* genotype. This is because being mild to the vector pays off especially when the parasite can monopolize exploitation of the host. Single infections are unlikely to be the rule, however, in *Plasmodium* parasites. This is because *Plasmodia* reproduce sexually and the fusion of gametocytes takes place exclusively in the vector! Clearly, for sexual reproduction (i.e. recombination) to generate new genotypes, multiple infection of the mosquito vectors is a prerequisite. Indeed, high rates of recombination have been recorded for *Plasmodium* populations in the field (Conway et al. 1999) and this suggests that multiple infection of vectors occurs.

Testing theoretical predictions may yield new insights and avenues in disease management. As a first example, consider the reduction of vector mobility by the application of bed nets that protect humans against mosquito bites and thereby malaria transmission. This method may have several – sometimes opposite – effects on the vector: (1) it may decrease multiple infection in the vector and thereby cause the parasite to be milder for the vector, and (2) it may alter relative mobility of mosquitoes vs. human hosts such that parasites become less harmful to humans than to mosquitoes. Whether bed nets can lower parasite diversity in the definitive host remains an open question. There is some empirical evidence showing that bed nets do not affect parasite diversity in the definitive host (Fraser Hurt et al. 1999). A second example of insights gained in disease management comes from the introduction of refractory vectors, i.e. transgenic mosquitoes that cannot transmit malaria parasites (e.g. Scott et al. 2002). Despite the negative effects of malaria parasites on vector fecundity, refractoriness probably did not evolve spontaneously, because of the costs involved in mounting immune responses (Schwartz and Koella 2001). Introduction of transgenic mosquitoes may therefore be successful provided refractoriness comes at a low cost. One may ask, however, how the introduction of transgenic mosquitoes would influence parasite virulence to the wild-type mosquitoes. If the mosquito population is regulated by density-dependent processes, introduction of transgenic mosquitoes will not increase their total population size and a mosquito bite is then on average less likely to transmit parasites. This could potentially lower the degree of multiple infection in the human host and the mosquito vector and thereby lower virulence of the parasite to either of the two hosts. Thus, compared to the wild-types, the transgenic mosquitoes have less to gain from being parasite-free, and according to a model by Boete and Koella (Boëte and Koella 2002) this will hamper replacement of wild mosquito populations by transgenic mosquitoes.

These two examples demonstrate the importance of evolutionary responses to control measures and it would be wise to take these potential responses into account when designing scenarios to combat disease.

Acknowledgements

We thank Arne Janssen, Andrew Read and Heather Ferguson for comments on this paper.

Appendix 1. A general ESS analysis for vectorborne diseases

A vectorborne parasite exploits two potential hosts on adjacent trophic levels, i.e. where one feeds on the other. Here, we consider a mosquito-host system where a parasite can exploit either of these two hosts for reproduction and for dispersal. This model serves as a basis to explore how patch structure and mobility can affect the parasite's virulence in the two hosts.

Single patch model

Suppose hosts fall into two categories, susceptible individuals (*S*) and infected individuals (*I*), as do mosquitoes (*U* and *V* respectively) (Fig. 1). Parasites can be transmitted through mosquito bites. This yields the following set of equations:

$$\frac{dI}{dt} = (1-a)\eta q(\gamma)SV - kI - \beta I - a\eta UI$$

$$\frac{dV}{dt} = \tau(\beta)\eta UI - k_p V - \gamma V$$

The key elements are the virulence to the two hosts to parasites (β and γ respectively) and how they translate into the two modes of transmission: (1) $\tau(\beta)$ is infectiousness of infected hosts to moquitoes, (2) $q(\gamma)$ is infectiousness of infected mosquitoes to hosts. In addition, parasite virulence can represent a dead-end in terms of transmission (for example in cerebral malaria where vital host tissue is exploited but no parasite transmission occurs). This is represented by *a*, the transmission-unrelated death rate, which is taken into account for prey but not for predator.

We assume that factors other than the parasite are regulating the population of uninfected predators (*U*). Thus, *U* is treated as a parameter, creating a one-resource model in which we examine the consequences of parasite virulences on uninfected prey (*S*). Optimal virulence of the parasite is found by invasion analysis (assuming that the predator and prey populations are at equilibrium). The un-invadible strain is the strain that minimizes *S**, the equilibrium susceptible population of hosts:

$$S^* = \frac{k+\beta+a\eta U}{\left\lceil \frac{(1-a)q(\gamma)\eta\tau(\beta)\eta U}{k_p+\gamma} \right\rceil}$$

The equilibrium susceptible population depends on the "in-host" virulence β only through the term:

$$\frac{k+\beta+a\eta U}{\tau(\beta)}$$

which has its minimum where

$$\tau'(\beta) = \frac{\tau(\beta)}{k+a\eta U+\beta}$$

$$\frac{q(\gamma)}{k_p+\gamma}$$

The equilibrium susceptible population depends on the "in-mosquito" virulence γ only through the factor

which has its maximum at

$$q'(\gamma) = \frac{q(\gamma)}{k_p + \gamma}$$

As we wish to focus on differences in virulence of the parasite to the host and the mosquito, as created by their different ecological roles, we assume their background mortalities to be equal ($k_p = k$). Under these conditions, the equation for the ESS virulence on mosquitoes matches that for hosts, so virulence is predicted to be equal in both.

Multi-patch model

Supposing interpatch transmission can also occur, we ask how it affects virulence to vector and host. All patches are occupied by both host and vector (but not the parasite) at all times, the infection rapidly reaches equilibrium after it has been introduced into a patch, and all patches have an equal probability of becoming extinct. To define the equilibrium, we must assume that the dynamics for the susceptible host follow logistic growth in the absence of vectorborne parasites, are subject to death from abiotic causes (k) and obey law of mass action in the vectorborne infection term that shifts susceptibles into the class of infecteds:

$$\frac{dS}{dt} = bS(1 - \frac{S}{N}) - kS - \eta q(\gamma)SV$$

The equilibria of the multiple patch model match those in the single patch model with the additional equation

$$V^* = \frac{b(1 - \frac{S^*}{N}) - k}{\eta q(\gamma)}$$

Patches are either occupied by parasites or not. Let p denote the fraction of infected patches, C the colonization rate of infected patches and E the patch extinction rate, then, a classic metapopulation model results:

$$\frac{dp}{dt} = Cp(1 - p) - Ep$$

Let m_I and m_V denote the per capita rate at which infected hosts and infected mosquitoes, respectively move parasites between patches.
Depending on whether parasites move between patches via vectors or via main hosts, the parasite colonization rate C is then

$$C = m_I I^*$$

$$or:$$

$$C = m_V V^*$$

To be consistent with the assumption of rapid approach to equilibrium, we assume that a patch harbours only a single parasite at a time and the superior competitor immediately replaces any inferior competitor (i.e. the superinfection assumption; see Mosquera and Adler 1998). The superinfection function $A(\tilde{S}^*, S^*)$ describes the rate at which a strain with equilibrium $\tilde{S}^*$ takes over a patch occupied by a strain with equilibrium S^* relative to the rate at which it takes over empty patches:

$$A(\tilde{S}^*, S^*) = \frac{(S^*)^n}{(\tilde{S}^*)^n + (S^*)^n}$$

Larger values of the parameter *n* produce steeper slopes of the superinfection function at $S\sim^*=S^*$ (Mosquera and Adler 1998).

The fraction $p\sim$ of patches occupied by an invading strain is

$$\frac{d\tilde{p}}{dt} = (-E + \tilde{C}(1-p) + \tilde{C}A(\tilde{S}^*, S^*)p - CA(S^*, \tilde{S}^*)p)\tilde{p}.$$

The goal is to find an uninvadible (or ESS) strain.

References

Beier, J.C., 1998. Malaria parasite development in mosquitoes. *Annual Review of Entomology,* 43, 519-543.

Boëte, C. and Koella, J.C., 2002. A theoretical approach to predicting the success of genetic manipulation of malaria mosquitoes in malaria control. *Malaria Journal,* 1 (1), 1-7. [http://www.malariajournal.com/content/1/1/3]

Bruce, M.C., Donnelly, C.A., Alpers, M.P., et al., 2000. Cross-species interactions between malaria parasites in humans. *Science,* 287 (5454), 845-848.

Chege, G.M.M. and Beier, J.C., 1990. Effect of *Plasmodium falciparum* on the survival of naturally infected Afrotropical *Anopheles* (Diptera: Culicidae). *Journal of Medical Entomology,* 27, 454-458.

Conway, D.J., Roper, C., Oduola, A.M.J., et al., 1999. High recombination rate in natural populations of *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences of the United States of America,* 96 (8), 4506-4511.

Day, T., 2001. Parasite transmission modes and the evolution of virulence. *Evolution,* 55 (12), 2389-2400.

Day, T., 2002. The evolution of virulence in vector-borne and directly transmitted parasites. *Theoretical Population Biology,* 62 (2), 199-213.

Dieckmann, U., Metz, J.A.J., Sabelis, M.W., et al. (eds.), 2002. *Adaptive dynamics of infectious diseases: in pursuit of virulence management*. Cambridge University Press, Cambridge, UK. Cambridge Studies in Adaptive Dynamics. Vol. 2.

Elliot, S.L., Adler, F.R. and Sabelis, M.W., submitted. How virulent should a parasite be to its vector? *Ecology*.

Ewald, P.W., 1994. *Evolution of infectious disease*. Oxford University Press, Oxford.

Faran, M.F., Turell, M.J., Romoser, W.S., et al., 1987. Reduced survival of adult *Culex pipiens* infected with Rift Valley Fever Virus. *Journal of Tropical Medicine and Hygiene,* 37 (2), 403-409.

Ferguson, H.M. and Read, A.F., 2002. Why is the effect of malaria parasites on mosquito survival still unresolved? *Trends in Parasitology,* 18 (6), 256-261.

Fraser Hurt, N., Felger, I., Edoh, D., et al., 1999. The epidemiology of multiple Plasmodium falciparum infections - 9. Effect of insecticide-treated bed nets on haemoglobin values, prevalence and multiplicity of infection with Plasmodium falciparum in a randomized controlled trial in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 93 Suppl. 1, S47-S51.

Gandon, S., Mackinnon, M.J., Nee, S., et al., 2001. Imperfect vaccines and the evolution of parasite virulence. *Nature,* 414 (6865), 751-756.

Ghosh, A., Edwards, M.J. and Jacobs-Lorena, M., 2000. The journey of the malaria parasite in the mosquito: Hopes for the new century. *Parasitology Today,* 16 (5), 196-201.

Hurd, H., Hogg, J.C. and Renshaw, M., 1995. Interactions between bloodfeeding, fecundity and infection in mosquitoes. *Parasitology Today,* 11, 411-416.

Kollien, A.H. and Schaub, G.A., 2000. The development of *Trypanosoma cruzi* in triatominae. *Parasitology Today,* 16 (9), 381-387.

Lam, K.S.K. and Marshall, I.D., 1968. Dual infections of *Aedes aegypti* with arboviruses. II. Salivary-gland damage by Semliki Forest Virus in relation to dual infections. *American Journal of Tropical Medicine and Hygiene,* 17, 637-644.

Maier, W.A., Becker-Feldman, H. and Seitz, H.M., 1987. Pathology of malaria-infected mosquitoes. *Parasitology Today,* 3, 216-218.

Mims, C.A., Day, M.F. and Marshall, I.D., 1966. Cytopathic effect of semliki forest virus in the mosquito *Aedes aegypti*. *American Journal of Tropical Medicine and Hygiene,* 15, 775-784.

Mosquera, J. and Adler, F.R., 1998. Evolution of virulence: a unified framework for coinfection and superinfection. *Journal of Theoretical Biology,* 195 (3), 293-313.

Power, A.G., 1992. Patterns of virulence and benevolence in insect-borne pathogens of plants. *Critical Reviews in Plant Sciences,* 11, 351-372.

Randolph, S.E., 1998. Ticks are not insects: Consequences of contrasting vector biology for transmission potential. *Parasitology Today,* 14 (5), 186-192.

Schwartz, A. and Koella, J.C., 2001. Trade-offs, conflicts of interest and manipulation in *Plasmodium*-mosquito interactions. *Trends in Parasitology,* 17 (4), 189-194.

Scott, T.W., Takken, W., Knols, B.G.J., et al., 2002. The ecology of genetically modified mosquitoes. *Science,* 298 (5591), 117-119.

Smith, T., Felger, I., Kitua, A., et al., 1999. The epidemiology of multiple *Plasmodium falciparum* infections - 7. Dynamics of multiple *Plasmodium falciparum* infections in infants in a highly endemic area of Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 93 Suppl. 1, S35-S39.

Snow, R.W., Craig, M., Deichmann, U., et al., 1999. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bulletin of the World Health Organization,* 77 (8), 624-640.

Taylor, L.H. and Read, A.F., 1997. Why so few transmission stages? Reproductive restraint by malaria parasites. *Parasitology Today,* 13 (4), 135-140.

Turell, M.J., Gargan, T.P. and Bailey, C.L., 1985. Culex pipiens (Diptera: Culicidae) morbidity and mortality associated with Rift Valley Fever Virus infection. *Journal of Medical Entomology,* 22, 332-337.

Welburn, S.C. and Maudlin, I., 1999. Tsetse-typanosome interactions: Rites of passage. *Parasitology Today,* 15 (10), 399-403.

Yan, G., Severson, D.W. and Christensen, B.M., 1997. Costs and benefits of mosquito refractoriness to malaria parasites: Implications for genetic variability of mosquitoes and genetic control of malaria. *Evolution,* 51 (2), 441-450.

Zieler, H. and Dvorak, J.A., 2000. Invasion in vitro of mosquito midgut cells by the malaria parasite proceeds by a conserved mechanism and results in death of the invaded midgut cells. *Proceedings of the National Academy of Sciences of the United States of America,* 97 (21), 11516-11521.