On the interpretation of age–prevalence curves for trypanosome infections of tsetse flies

M. E. J. WOOLHOUSE[1]* *and* J. W. HARGROVE[2]

[1] *Wellcome Trust Centre for the Epidemiology of Infectious Diseases, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS*
[2] *ODA IPMI, Tsetse and Trypanosomiasis Control Branch, Box CY52, Causeway, Harare, Zimbabwe*

(*Received 11 April 1997; revised 28 July 1997; accepted 1 August 1997*)

SUMMARY

Epidemiological models are used to analyse 8 published data sets reporting age–prevalence curves for trypanosome infections of the tsetse fly *Glossina pallidipes*. A model assuming a fixed maturation period and a rate of infection which is independent of fly age is adequate for *Trypanosoma vivax*-type infections, explaining 98% of observed variance in prevalence by site and age, allowing that the rate of infection may be site dependent. This model is not adequate for *T. congolense*-type infections and the fit can be improved by allowing (i) the rates of infection to decline with age (although non-teneral flies remain susceptible), (ii) a fraction of resistant flies, which may vary between sites, (iii) increased mortality of infected flies and (iv) variation in the maturation period. Models with these features can explain up to 97% of observed variance. Parameter estimates from published experimental data suggest that all may contribute in practice but that (i) and/or (ii) are likely to be the most important.

Key words: epidemiology, *Glossina pallidipes*, mathematical models, *Trypanosoma congolense*, *Trypanosoma vivax*.

INTRODUCTION

The prevalence of mature trypanosome infections of tsetse flies in the field has been widely reported to increase with age (e.g. Harley, 1966; Rogers & Boreham, 1973; Ryan *et al*. 1982; Woolhouse, Hargrove & McNamara, 1993; Woolhouse *et al*. 1994). The shape of the age–prevalence curve provides information on the biological process underlying the epidemiology of the host–parasite interaction (Woolhouse, 1989) and several aspects of the tsetse–trypanosome interaction may have an impact on the shape of the age–prevalence curves reported in field studies. (i) Age-dependent rate of infection. Several laboratory studies have reported that rates of infection decline in older flies, e.g. for *T. brucei* infection (Jordan, 1976) and *Trypanosoma congolense* infection of *Glossina morsitans* (Moloo & Shaw, 1989), and several studies have suggested that teneral flies are most susceptible to infection (Welburn & Maudlin, 1992), especially with *T. brucei* (see Maudlin, 1991). (ii) Differential susceptibility. Several studies have suggested that not all flies are susceptible to infection, possibly reflecting a sex-linked genetic factor or maternally inherited rickettsia-like organisms (RLOs) (see Maudlin, 1991). (iii) Mortality of infected flies. One study has reported that *G.m. morsitans* with mature *T. congolense* infections suffer increased mortality (Nitcheman, 1988), and trypanosome infections of *G. longipalpis* may result in poorer nutritional condition (Ryan, 1984). (iv) Variation in maturation period. This variation has been quantified for *T. brucei* and *T. congolense* infections of *G. m. morsitans* (Dale *et al*. 1995).

Here, age–prevalence data for *T. vivax* and *T. congolense* infections of tsetse from 8 field studies in sub-Saharan Africa are analysed and the fits of different mathematical models to these data are compared, the models representing the different epidemiological processes listed above. This type of analysis is intended to aid the interpretation of the data, indicating which processes are consistent with the epidemiological evidence and providing quantitative estimates of the magnitude of the effects that would be required to produce observed patterns.

METHODS

Field data

Age–prevalence data were taken from published studies of female *G. pallidipes* aged by ovarian dissection. Following Challier (1965) 8 ovarian categories, 0 to 7, were recognized. Studies with ageing by wing fray only were excluded; although

* Corresponding author: Centre for Veterinary Tropical Medicine, University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG. Tel: and Fax: +0131 650 6264. E-mail: Mark.Woolhouse@ed.ac.uk

https://doi.org/10.1017/S0031182097002047 Published online by Cambridge University Press

Table 1. Summary of data sets

Source	Study location	Sample size (n)	Prevalence (%)	
			Tc-type	*Tv*-type
(a) Woolhouse *et al.* (1993)	Zambezi Valley, Zimbabwe	5690	2·53	3·22
(b) Woolhouse *et al.* (1994)	Luangwa Valley, Zambia	3152	2·98	6·12
(c) Harley (1966)	Lugala, Uganda	1445	4·64	25·4
(d) Tarimo *et al.* (1985)	Diani, Kenya	2680	4·48	2·72
(e) Tarimo *et al.* (1985)	Ukunda and Muhaka, Kenya	1960	8·62	1·53
(f) Tarimo *et al.* (1985)	Simba Hills and Mwalewa, Kenya	4876	3·81	3·40
(g) Tarimo Nesbitt *et al.* (1991)	Lambwe Valley, Kenya	3811	2·26	10·5
(h) Bealby *et al.* (1996)	Luangwa Valley, Zambia	7645	1·05	2·05

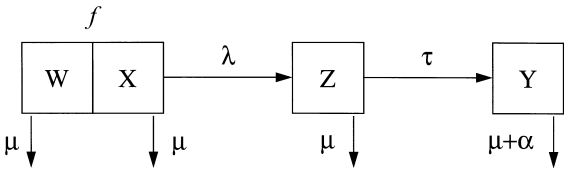

Fig. 1. Diagrammatic representation of the compartmental model described in the main text. W represents the number of non-susceptible flies, X represents uninfected, susceptible flies, Z represents flies with immature infections, and Y represents flies with mature infections. The fractions of susceptible and non-susceptible flies at age zero is set by the parameter f. Susceptible flies become infected at the *per capita* rate λ. Infections mature after the period τ. All flies die at the *per capita* rate μ. Flies with mature infections suffer an additional *per capita* mortality rate α.

wing fray and ovarian ages are correlated in individual studies (Hargrove, 1990; Bealby, Connor & Rowlands, 1996), wing fray ageing alone is not sufficiently reliable for these analyses. In each study the prevalence and identity of mature infections were determined by dissection of the mouthparts and midgut: trypanosomes were classified as *T. congolense*-type (trypanosomes present in the mouthparts and midgut) or *T. vivax*-type (mouthparts only). Some infections were also identified as *T. brucei*-type (trypanosomes present in the salivary glands) but these data are sparse and are not considered here. Using these criteria 8 data sets from 6 studies of both *T. vivax*-type and *T. congolense*-type infections were identified; details are given in Table 1. Sample sizes ranged from 1445 to 7645 and overall prevalences of infection from 1·5 to 25·4% for *T. vivax*-type and from 1·1 to 8·6% for *T. congolense*-type.

Ovarian categories 0 and 1 are taken to correspond to 0–8 and 9–16 days old respectively and ovarian categories 2–7 to correspond to additional intervals of 9 days from 17–25 to 62–70 days old respectively. There may be biases in this approach because flies in the third and subsequent ovarian cycles are allocated to ovarian categories 4–7 rather than 8–11 and beyond (Hargrove, 1990). However, the numbers of flies surviving beyond 70 days is low in at least some cases (Woolhouse *et al.* 1993). The effects of any such bias are discussed below.

Model development

Model structure is shown diagrammatically in Fig. 1. The tsetse population is divided into 4 compartments: uninfected, susceptible flies, denoted by the variable X; flies with immature infections, denoted Z; flies with mature infections, denoted Y; and a compartment representing resistant flies, denoted W. Uninfected, susceptible flies become infected at the *per capita* rate λ, which may vary between sites and with fly age. Flies with immature infections become flies with mature infections after a maturation period τ, which may have a variance. All flies die at the *per capita* rate μ, and flies with mature infections may suffer an additional *per capita* mortality rate α. Assuming steady-state conditions, the rates of change of X, Z and Y with respect to fly age, a, are given by the coupled differential equations:

$$\frac{dX}{da} = -(\lambda(a)+\mu)\,X(a)$$

$$\frac{dZ}{da} = \lambda(a)\,X(a) - \theta(a)\,\lambda(a-\tau) \times \exp[-\mu\tau]\,X(a-\tau) - \mu Z(a)$$

$$\frac{dY}{da} = \theta(a)\,\lambda(a-\tau)\exp[-\mu\tau]\,X(a-\tau) - (\mu+\alpha)\,Y(a),$$

https://doi.org/10.1017/S0031182097002047 Published online by Cambridge University Press

where $\theta(a)$ is a step function returning a value of 0 if $a \leqslant \tau$ and 1 if $a > \tau$. Initial conditions are: $X(0) = fN(0)$ for $0 < f \leqslant 1$ where f is the fraction susceptible to infection and $N(0)$ is the number of flies born into the cohort; and $Z(0) = Y(0) = 0$.

These equations can be solved to give the fraction of flies with mature infections at age a, $y(a) = Y(a)/N(a)$ where $N(a)$ is the number surviving to age a and is given by $N(a) = X(a) + Y(a) + Z(a) + W(a)$ where $W(a) = (1-f)N(0)\exp[-\mu a]$. In all cases this gives $y(a) = 0$ for $a \leqslant \tau$. Special cases of this general model correspond to the different epidemiological processes of interest here. Six specific models are considered.

Model 1. Basic model with $\lambda(a) = \lambda, f = 1$ and $\alpha = 0$. This gives:

$$y(a) = 1 - \exp[-\lambda(a-\tau)].$$

Model 2. Rate of infection declines with age. Different forms of this decline are possible, here an exponential function is assumed so that $\lambda(a) = \lambda \exp[-ba]$ (where b is the rate of decline of the rate of infection with age). This gives:

$$y(a) = 1 - \exp\left\{-\frac{\lambda}{b}(1 - \exp[-b(a-\tau)])\right\}.$$

Model 3. Resistant fraction, $f < 1$. This gives:

$$y(a) = f\{1 - \exp[-\lambda(a-\tau)]\}.$$

Model 4. A variation of model (3) which allows the fraction of susceptible flies, f, to vary between sites.

Model 5. Additional mortality of flies with mature infections, $\alpha < 0$. This gives:

$$y(a) = \frac{\lambda}{\lambda - \alpha \exp[(\alpha-\lambda)(a-\tau)]} \times \{1 - \exp[(\alpha-\lambda)(a-\tau)]\}.$$

Model 6. Flies susceptible only at the first feed (taken soon after birth) with variable maturation period. Different distributions of the maturation period are possible, here it is assumed that:

$$y(a) = \lambda_0\{1 - \exp[-(\gamma a)^\beta]\},$$

where λ_0 is the fraction of first feeds that results in infection.

Model fitting

Models (1)–(6) were fitted separately to data for *T. vivax*-type and *T. congolense*-type infections. For each trypanosome type all data sets were included in a single analysis, but the *per capita* rate of infection, λ, was allowed to vary between data sets. For model (4) the fraction of susceptible flies, f, was also allowed to vary between data sets. Fly age was taken as the mid-point of the range represented by the ovarian category. However, because preliminary analysis indicated that the maturation period, τ, could be up to 17 days, the small number of infections observed in flies in ovarian categories 0 and 1 were unlikely to have occurred in flies at the mid-point age. These age categories were therefore not included for model fitting, although they were considered in interpreting model fit.

Model fitting was carried out using a weighted least squares method, DUD in the SAS statistical package. The fraction of flies with mature infections, p, in each age class for each data set was weighted by the reciprocal of the binomial variance, i.e. $1/np(1-p)$ where n is the sample size (see Williams & Dye, 1994). Coefficients of determination, R^2, are given as an indicator of goodness-of-fit. F-values are used to test the statistical significance of the added parameters in the more complex models, as compared with the basic model.

RESULTS

The age–prevalence curves for *T. vivax*-type and *T. congolense*-type infections obtained from the field studies are shown in Figs 2 and 3. The fits of models (1)–(6) to these data are reported in Table 2.

For *T. vivax*-type infections the basic model explains 97·8 % of the variance in prevalences by age and site. This model gives a best estimate of the maturation period of $\tau = 10{\cdot}5$ days, and estimates of the rate of infection ranging from $\lambda = 0{\cdot}053$ to 1·1/fly/100 days. None of the models incorporating additional parameters give a significant improvement in fit (Table 2A). Model (6) explains 98·1 % of the variance: the estimated probabilities of infection at the first bloodmeal range from 0·03 to 0·52 and the estimated values of β and γ correspond to a mean maturation period of 38·8 with a standard deviation of 20·3 days.

For *T. congolense*-type infections the basic model explains 93·5 % of the variance. This model gives an estimate of the maturation period of $\tau = 8{\cdot}0$ days, and estimates of the rate of infection ranging from $\lambda = 0{\cdot}028$ to 0·33/fly/100 days. In this case the fit is improved by incorporating additional parameters in the model (Table 2B). The best model with 1 additional parameter, model (3), gives the fraction of flies susceptible to infection as $f = 0{\cdot}18$, and explains 94·7 % of the variance. The models allowing either that there is additional mortality due to infection, model (5), or that the rate of infection declines with age, model (2), also all give significant improvements in fit over the basic model. It is not possible to discriminate between the fits of models (2), (3) and (5). These models give higher estimates of the maturation period, up to $\tau = 17{\cdot}6$ days, and higher estimates of the rates of infection than the basic model. In each case estimates of individual par-

https://doi.org/10.1017/S0031182097002047 Published online by Cambridge University Press

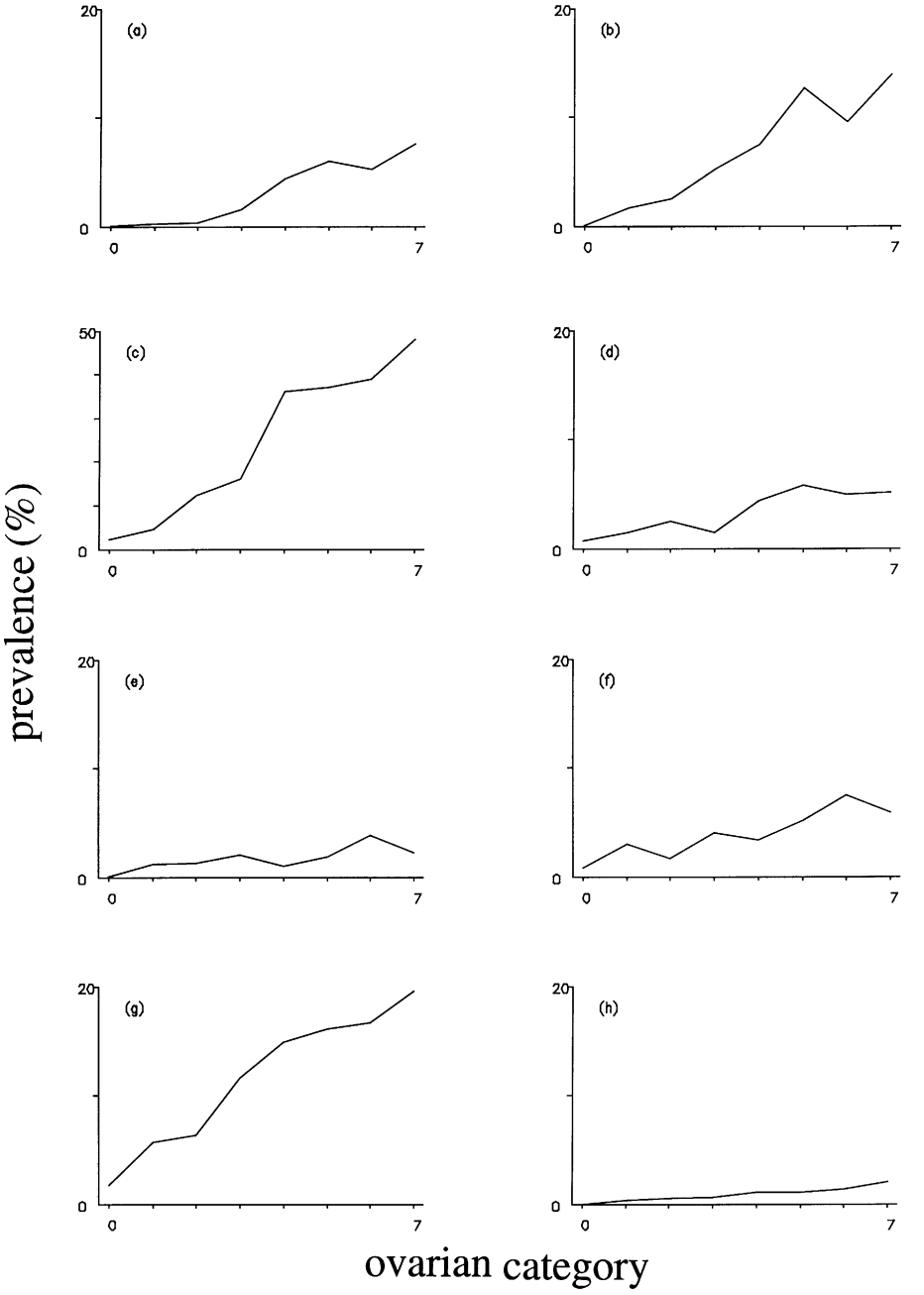

Fig. 2. Age–prevalence data for *Trypanosoma vivax*-type infections of female *Glossina pallidipes*. Horizontal axes show ovarian categories 0–7. Vertical axes show prevalence of infection in units of 10% – note different scale on (c). Graphs (a)–(h) show data from the corresponding studies listed in Table 1.

ameter values should be treated with some caution as these estimates tend to be correlated. Model (4), which allows site-specific fractions of susceptible flies, provides a further significant improvement in fit over model (3), explaining 97·0% of the variance. Model (6) explains 94·7% of the variance: the estimated probabilities of infection at the first bloodmeal range from 0·02 to 0·17 and the estimated value of β and γ correspond to a mean maturation period of 34·2 days with a standard deviation of 13·0 days.

The differences between the age–prevalence curves predicted by the different models are illustrated in Fig. 4. The basic model predicts a near linear increase in prevalence with age, unless prevalences are very high (Fig. 4A). The model with infection occurring only in the youngest flies and variance in the maturation period predicts sigmoid age–prevalence curves (Fig 4B). The model with a resistant fraction of flies predicts more convex age–prevalence curves, especially if prevalences are high (Fig. 4C). The model with additional mortality of infected flies also predicts more convex curves, but this also applies when prevalences are low (Fig. 4D); the model with declining susceptibility with age behaves similarly.

https://doi.org/10.1017/S0031182097002047 Published online by Cambridge University Press

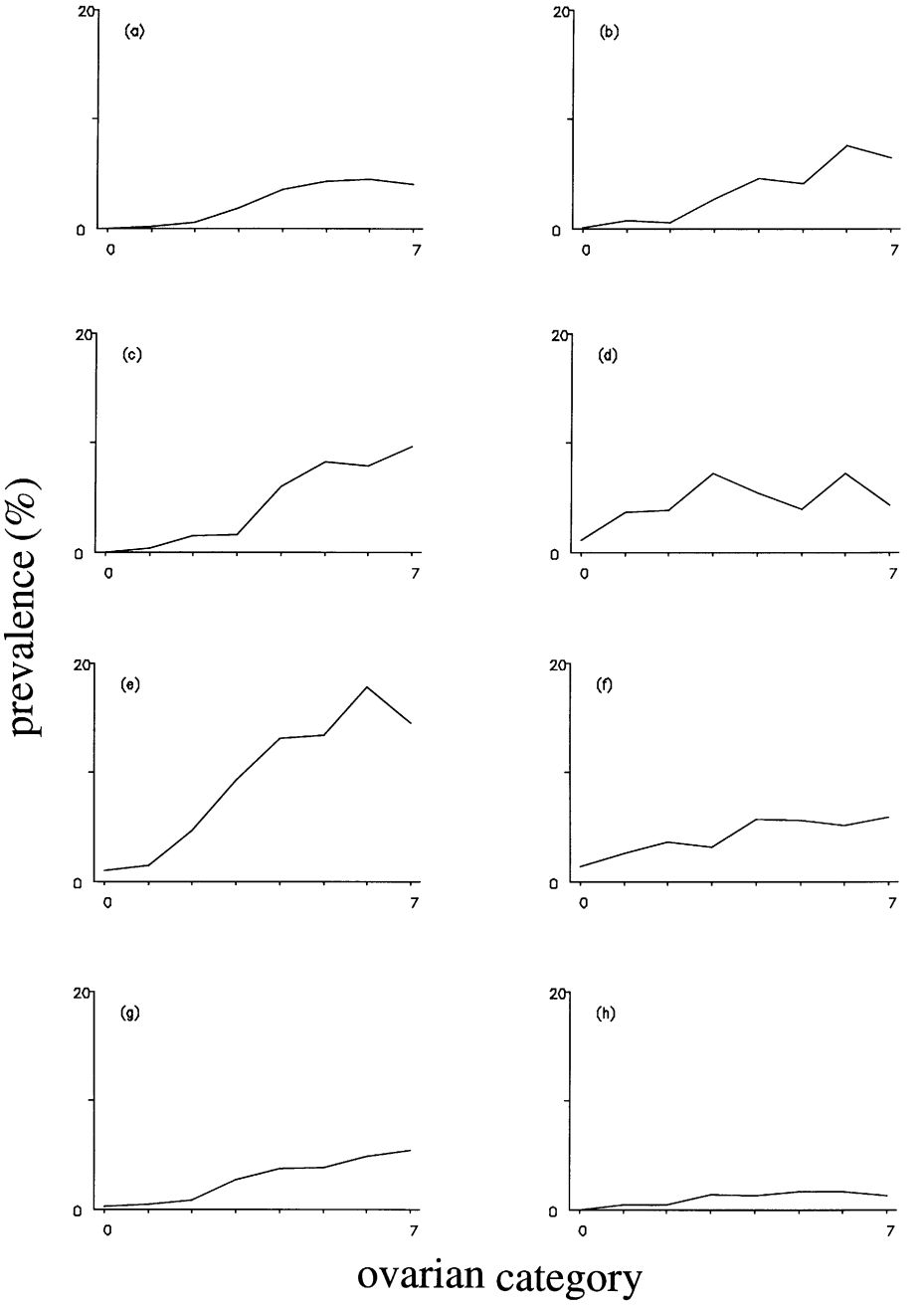

Fig. 3. Age–prevalence data for *Trypanosoma congolense*-type infections of female *Glossina pallidipes*. Horizontal axes show ovarian categories 0–7. Vertical axes show prevalence of infection in units of 10%. Graphs (a)–(h) show data from the corresponding studies listed in Table 1.

DISCUSSION

For *T. vivax*-type infections the observed age–prevalence data are well explained by a model which incorporates only a constant rate of infection and a fixed period for maturation of the infection. For *T. congolense*-type infections, however, there is clear evidence that the observed age–prevalence curves are not adequately described by a model assuming a constant rate of infection and a fixed maturation period. The curves are significantly convex, which suggests that at least 1 of the following epidemiological processes is important: (i) declining rate of infection with age, (ii) a resistant fraction of flies, (iii) additional mortality of infected flies and (iv) variation in the maturation period. In principle, it is possible to distinguish between these because they have different effects on the shapes of the age–prevalence curve though, in practice, the data are not adequate to do so. However, a significantly better fit than all these models is obtained using the model allowing the fraction of susceptible flies to vary between sites. This suggests that site-specific effects, beyond differences in the rate of infection, are important.

Several caveats should be noted. First, the effect of classifying any flies in their third or later ovarian cycle (categories 8–11 and beyond) as being in their second ovarian cycle (categories 4–7) will be to

https://doi.org/10.1017/S0031182097002047 Published online by Cambridge University Press

Table 2. Comparison of model fits

(R^2 is the coefficient of determination. F-values and degrees of freedom refer to the addition of 1 extra parameter to the basic model, with corresponding P values, except that model (4) is compared to model (3) and has 7 additional parameters (8 site-specific resistant fractions rather than a pooled resistant fraction). No F-value is calculated for model (6) as this has a different form from the basic model. The range of estimated λ values is shown, and of estimated f values for model (4). τ is the developmental period. Additional parameters are defined in Fig. 1. The time unit is 1 day.)

Model	R^2	F (D.F.)	P	$\lambda \times 100$	$\tau \pm$ S.E.	Additional parameter(s) $\pm$ S.E.
A *Trypanosoma vivax*-type infections						
1	0·978	—		0·053–1·1	$10{\cdot}5 \pm 1{\cdot}6$	—
2	0·979	2·0 (1,38)	$> 0{\cdot}10$	0·066–1·5	$13{\cdot}3 \pm 2{\cdot}2$	$b = 0{\cdot}0099 + 0{\cdot}0074$
3	0·978	0·3 (1,38)	$> 0{\cdot}50$	0·059–1·3	$10{\cdot}1 \pm 2{\cdot}7$	$f = 0{\cdot}88 \pm 0{\cdot}34$
5	0·980	2·1 (1,38)	$> 0{\cdot}10$	0·075–1·6	$13{\cdot}4 \pm 1{\cdot}8$	$\alpha = 0{\cdot}016 \pm 0{\cdot}011$
6	0·981	—		2·9–52	—	$\beta = 1{\cdot}9 \pm 0{\cdot}6$, $\gamma = 0{\cdot}023 \pm 0{\cdot}009$
B *Trypanosoma congolense*-type infections						
1	0·935	—		0·028–0·33	$8{\cdot}0 \pm 2{\cdot}4$	—
2	0·942	4·6 (1,38)	$< 0{\cdot}05$	0·10–0·94	$17{\cdot}6 \pm 1{\cdot}2$	$b = 0{\cdot}044 \pm 0{\cdot}010$
3	0·947	8·6 (1,38)	$< 0{\cdot}01$	0·21–4·6	$14{\cdot}5 \pm 1{\cdot}8$	$f = 0{\cdot}18 \pm 0{\cdot}02$
4	0·970	5·3 (7,31)	$< 0{\cdot}001$	0·86–28	$15{\cdot}9 \pm 1{\cdot}3$	$f = 0{\cdot}019$–$0{\cdot}26$
5	0·942	4·7 (1,38)	$< 0{\cdot}05$	0·068–0·65	$15{\cdot}2 \pm 2{\cdot}6$	$\alpha = 0{\cdot}027 \pm 0{\cdot}014$
6	0·947	—		1·9–17	—	$\beta = 2{\cdot}8 \pm 0{\cdot}4$, $\gamma = 0{\cdot}026 \pm 0{\cdot}001$

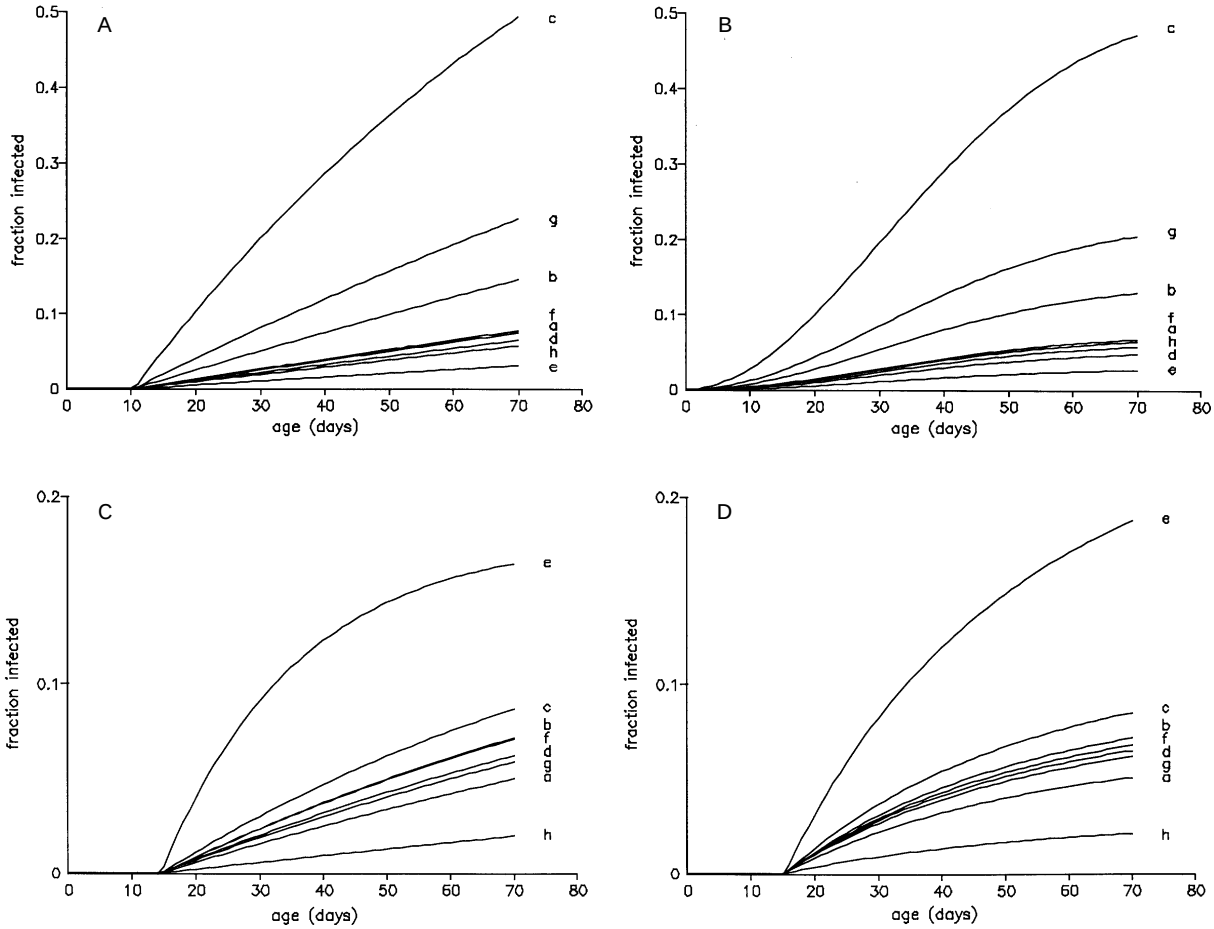

Fig. 4. Age–prevalence curves predicted by different epidemiological models. (A) The basic model, model (1), for *Trypanosoma vivax*-type infections. Curves correspond to parameter values estimated for each of the 8 field studies (see Tables 1 and 2). (B) As (A) for model with infection of youngest flies only and variance in the maturation period, model (6), for *T. vivax*-type infections. (C) As (A) for model with only a fraction of flies susceptible, model (3), for *T. congolense*-type infections. (D) As (A) for model with additional mortality of flies with mature infections, model (5), *T. congolense*-type infections.

https://doi.org/10.1017/S0031182097002047 Published online by Cambridge University Press

reduce the apparent convexity of the age–prevalence curve, provided such flies have the same or higher prevalences than true second-cycle flies. This effect will, of course, be small if the number of flies in category 8 or above is small, as in at least some of these studies (Woolhouse *et al*. 1993). Second, the best estimates of the maturation periods for both infection types do not predict any mature infections in category 0 flies, although several of the 8 field studies report such infections. This implies variation in the maturation period, so that some infections do mature within 8 days or less. Third, the analysis assumes steady-state conditions. This condition is met for at least some studies: Woolhouse *et al*. (1993, 1994) found no change in the shape of the age–prevalence curves over periods of 12 months or more. However, non-steady-state conditions, such as rapidly rising or falling rates of infection, will affect the age–prevalence curve, leading to increased or decreased convexity respectively (Woolhouse, 1989). Fourth, model fits are sensitive to the functions assumed, particularly for the change in infection rate with age and for the distribution of the maturation period. Other functions may give better fits.

The estimated maturation periods for both infection types are consistent with earlier estimates (Davies, 1977). The estimated rates of infection are highly variable. Assuming a bloodmeal is taken on average every 4 days (Rogers, 1988) these correspond to a successful infection occurring once every 23–472 bloodmeals for *T. vivax*-type infections and once every 17–301 bloodmeals for *T. congolense*-type (for the best fit model and noting that the rates given in Table 2B are per susceptible fly), presumably reflecting variation in the infectiousness of the vertebrate host population.

Results from experimental studies provide some independent evidence for distinguishing between possible models. The parameter estimates for the model assuming a decline in the rate of infection with age correspond to a reduction of 50% at 16 days old and of 90% at 52 days old. This is broadly consistent with data from Welburn & Maudlin (1992) showing that non-teneral outbred *G. m. morsitans* are only one-third as susceptible to infection as teneral flies. Both these experimental results and the analysis of field data suggest that older flies can become infected (Maudlin, 1991). The parameter estimates for the model assuming additional mortality of flies with mature infections correspond to an additional mortality rate of 0·027/fly/day. Data from Nitcheman (1988) give an additional mortality rate of 0·007/fly/day, substantially lower but obtained under laboratory conditions. The parameter estimates for the model assuming infection only in the youngest flies and variance in the maturation period correspond to a mean maturation period of up to 34 days with standard deviation 13 days. In contrast, data from Dale *et al*. (1995), in a study designed to test this model, give mean maturation periods of 12–16 days with standard deviations 5–6 days, clearly not adequate to explain the field data by this mechanism alone. Parameter estimates for the model assuming a resistant fraction of flies correspond to 18% flies being susceptible to infection. If the resistant fraction is allowed to vary between sites a range of 2–26% susceptible is obtained. Resistance may be associated with genetic factors or with the absence of symbiotic RLOs; the frequency of RLO infections is known to be highly variable in the field (Maudlin, 1991). Support for this model comes from an analysis reporting that the high degree of aggregation of different *T. congolense*-type infections in the Zambezi Valley, Zimbabwe could be explained if less than 10% flies were susceptible (Woolhouse *et al*. 1996); the best estimate of the fraction susceptible at that site from the present analysis is 6·3%.

A full understanding of these epidemiological processes is necessary to identify the factors determining the prevalence of infection of tsetse, and hence of trypanosome challenge to vertebrate hosts. Which processes are important also influences how the prevalence is affected by changes in rates of infection of tsetse resulting from efforts at trypanosmiasis control. It will not be possible to predict fully the long-term impact of control measures without knowledge of the dynamics of trypanosome infections of tsetse.

M.E.J.W. gratefully acknowledges the support of the Royal Society. Field work in Zimbabwe was supported by the ODA (RS4259) and the IAEA (TC Zim/5/004).

REFERENCES

BEALBY, K. A., CONNOR, R. J. & ROWLANDS, G. J. (1996). *Trypanosomiasis in Goats in Zambia*. ILRI, Kenya.

CHALLIER, A. (1965). Amélioration de la méthode de détermination de l'âge physiologique des glossines. Etudes faites sur *Glossina palpalis palpalis* Vanderplank, 1949. *Bulletin de la Société de Pathologie Exotique* **58**, 250–259.

DALE, C., WELBURN, S. C., MAUDLIN, I. & MILLIGAN, P. J. M. (1995). The kinetics of maturation of trypanosome infections of tsetse. *Parasitology* **111**, 187–192.

DAVIES, H. (1977). *Tsetse Flies in Nigeria*, 3rd Edn. Oxford University Press, Ibadan.

HARGROVE, J. W. (1990). Age-dependent changes in the probabilities of survival and capture of the tsetse, *Glossina morsitans morsitans* Westwood. *Insect Science and its Application* **11**, 323–330.

HARLEY, J. M. B. (1966). Further studies on age and trypanosome infection rates in *Glossina pallidipes* Aust., *G. palpalis fuscipes* Newst. and *G. brevipalpis* Newst. in Uganda. *Bulletin of Entomological Research* **57**, 459–477.

JORDAN, A. M. (1976). Tsetse flies as vectors of trypanosomes. *Veterinary Parasitology* **2**, 143–152.

MAUDLIN, I. (1991). Transmission of African trypanosomiasis: interactions among tsetse immune system, symbionts, and parasites. *Advances in Disease Vector Research* **7**, 117–148.

MOLOO, S. K. & SHAW, M. K. (1989). Rickettsial infections of midgut cells are not associated with susceptibility of *Glossina morsitans centralis* to *Trypanosoma congolense* infection. *Acta Tropica* **46**, 223–227.

NITCHEMAN, S. (1988). Comparison des longévités des glossines (*Glossina morsitans morsitans* Westwood, 1850) infectées par les trypanosomes (*Trypanosoma nannomonas congolense* Broden, 1904) et des glossines saines. *Annales de Parasitologie Humaine et Comparée* **63**, 163–164.

ROGERS, D. J. (1988). A general model for the African trypanosomiases. *Parasitology* **97**, 193–212.

ROGERS, D. & BOREHAM, P. F. L. (1973). Sleeping sickness survey in the Serengeti Area (Tanzania) 1971. II. The vector role of *Glossina swynnertoni* Austen. *Acta Tropica* **30**, 24–35.

RYAN, L. (1984). The effect of trypanosome infection on a natural population of *Glossina longipalpis* Wiedemann (Diptera: Glossinidae) in Ivory Coast. *Acta Tropica* **41**, 355–359.

RYAN, L., KUPPER, W., GOFF, S. L., MOLYNEUX, D. H. & CLAIR, M. (1982). Differences in rates of acquisition of trypanosome infections between *Glossina* species in the field. *Annales de la Société Belge de Médecine Tropicale* **62**, 291–300.

TARIMO, S. A., SNOW, F. W., BUTLER, L. & DRANSFIELD, R. (1985). The probability of tsetse acquiring trypanosome infection from a single blood meal in different localities in Kenya. *Acta Tropica* **42**, 199–207.

TARIMO NESBITT, S. T., NJAU, B. C. & OTIENO, L. H. (1991). Epizootiology of trypanosomiasis in Lambwe Valley, Kenya, East Africa. *Insect Science and its Application* **12**, 379–384.

WELBURN, S. C. & MAUDLIN, I. (1992). The nature of the teneral state in *Glossina* and its role in the acquisition of trypanosome infections in tsetse. *Annals of Tropical Medicine and Parasitology* **86**, 529–536.

WILLIAMS, B. G. & DYE, C. (1994). Maximum likelihood for parasitologists. *Parasitology Today* **10**, 489–493.

WOOLHOUSE, M. E. J. (1989). On the interpretation of age–prevalence curves for schistosome infections of host snails. *Parasitology* **99**, 47–56.

WOOLHOUSE, M. E. J., HARGROVE, J. W. & MCNAMARA, J. J. (1993). Epidemiology of trypanosome infections of the tsetse fly *Glossina pallidipes* in the Zambezi Valley. *Parasitology* **106**, 479–485.

WOOLHOUSE, M. E. J., BEALBY, K., MCNAMARA, J. J. & SILUTONGWE, J. (1994). Trypanosome infections of the tsetse fly *Glossina pallidipes* in the Luangwa Valley, Zambia. *International Journal for Parasitology* **24**, 987–993.

WOOLHOUSE, M. E. J., MCNAMARA, J. J., HARGROVE, J. W. & BEALBY, K. A. (1996). Distribution and abundance of trypanosome (subgenus *Nannomonas*) infections of the tsetse fly *Glossina pallidipes* in southern Africa. *Molecular Ecology* **5**, 11–18.