Trypanosomes, fleas and field voles: ecological dynamics of a host-vector–parasite interaction

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SUMMARY

To investigate the prevalence of a flea-borne protozoan (*Trypanosoma* (*Herpetosoma*) *microti*) in its field vole (*Microtus agrestis*) host, we monitored over a 2-year period a range of intrinsic and extrinsic parameters pertaining to host demographics, infection status and vector (flea) prevalence. Generalized Linear Mixed Modelling was used to analyse patterns of both flea and trypanosome occurrence. Overall, males of all sizes and ages were more likely to be infested with fleas than their female counterparts. Flea prevalence also showed direct density dependence during the winter, but patterns of density dependence varied amongst body mass (age) classes during the summer. Trypanosome prevalence did not vary between the sexes but was positively related to past flea prevalence with a lag of 3 months, with the highest levels occurring during the autumn season. A convex age-prevalence distribution was observed, suggesting that individuals develop a degree of immunity to trypanosome infection with age and exposure. An interaction between age and whether the individual was new or recaptured suggested that infected animals are less likely to become territory holders than their uninfected counterparts.

Key words: *Trypanosoma microti*, *Microtus agrestis*, field vole, vector borne, Siphonaptera, acquired immunity, natural population, sex-bias, Kielder Forest.

INTRODUCTION

Field voles (*Microtus agrestis*), like other rodents, are exposed to ectoparasites such as fleas (*Siphonaptera*) and ticks (*Ixodida*) and, as a result, are commonly infected with parasites associated with vector-borne transmission (e.g. the protozoan *Trypanosoma microti* Laveran and Pettit, 1909; Molyneux, 1968; Noyes *et al*. 2002). As endemic infections they are widely believed to be of little importance in host population dynamics and have received relatively little attention from ecologists. However, an increasing number of vector-borne parasites are becoming recognized as potentially important zoonotics (Daszak, Cunningham and Hyatt, 2000; Williams *et al*. 2002), and there is a need for a greater understanding of how infections are maintained and transmitted under natural conditions (Morner *et al*. 2002). Although *T. microti* is non-pathogenic to humans it does offer a model system to investigate the dynamics of a flea-transmitted disease within a naturally occurring wildlife population. More specifically, it offers the opportunity to determine whether the dynamics of a flea-borne pathogen are directly linked to the dynamics of its vector, or whether there are additional factors that are important in understanding both temporal variation and host heterogeneity in the maintenance and transmission of infection.

T. microti is a host-specific stercorarian trypanosome of the subgenus *T.* (*Herpetosoma*) (but see Noyes *et al*. 2002). Many rodent trypanosomes are considered to be of low pathogenicity (Hoare, 1972), but there is evidence for anaemia resulting from infection in microtines, and a condition-dependent or stress-related increase in virulence in some insect-trypanosome relationships (Wiger, 1977; Brown, Loosli and Schmid-Hempel, 2000). The main route of transmission from infected flea to vertebrate host is via contamination of a bite wound or feeding site by the infective-stage metatrypanosomes shed in the faeces of the feeding flea. A secondary route of infection is thought to be via the ingestion of contaminated fleas or flea faeces by the vole during grooming, which subsequently leads to infective metatrypanosomes penetrating the oral mucous membrane and entering the bloodstream (Hoare, 1972). The reproductive phase of *T. microti* within the field vole host takes approximately 7–13 days under laboratory conditions, including an incubation period of 6–9 days (Molyneux, 1969). Once the infection becomes established it lasts between 23 and 74 days, with a patent period typically showing a rapid rise in parasitaemia ($\geqslant$10 000 parasites/μl) then a steady decline towards the end of infection (Hoare, 1972).

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Although the trypanosomes of zoonotic and economic importance are particularly well studied (Barrett *et al*. 2003), surprisingly little is known of the ecology of the rodent trypanosomes within their natural environment (Wita, Karbowiak and Czaplinska, 2003). For example Healing (1981) concluded that trypanosome infection was more prevalent in younger rodents and was related to host density, but was not affected by extrinsic factors such as season, whereas Turner (1986) suggested that infection was predominantly found amongst older rodents and followed a seasonal pattern, with high prevalence during the summer and autumn. More recently, Bajer *et al*. (2001) and Pawelczyk *et al*. (2004) found that the variation in prevalence of haemoparasites, including trypanosomes in bank voles (*Clethrionomus glareolus*) and common voles (*Microtus arvalis*), was determined by temporal and seasonal factors and their interactions while intrinsic factors such as age and sex played only a minor role.

However, despite their crucial role in maintaining and transmitting infection, few studies on trypanosome dynamics within natural populations have taken into account the ecology of the vector species involved. To our knowledge, no other longitudinal study has considered the effects of both host and vector with regards to trypanosome dynamics. The aim of the present study, therefore, using 2 years of field data from 2 sites, was first to seek to understand the prevalence of fleas occurring on field vole hosts using characteristics such as host density, age, sex and extrinsic factors such as season. Subsequently, the aim was to seek to understand the prevalence and distribution of trypanosome infection within the populations using variables describing both flea and field vole dynamics together with the effects of seasonal variation.

MATERIALS AND METHODS

Study site

The study was carried out within two clear-cut areas in Kielder Forest, Northumberland, England (55° 13′ N, 2° 33′ W), a large (~600 km²) man-made forest with Sitka spruce (*Picea sitchensis*) and Norway spruce (*Picea abies*) providing the bulk of the harvest. Timber extraction produces isolated clear-cut areas ranging from 5 to 50 ha over a 50-year rotational period. Following 2–3 years of 'successional' growth the clear-cut areas become dominated by *Deschampsia cespitosa* and *Juncus effuses* and thus become suitable habitat for field voles. Further regeneration leads to a low scrub/thicket stage that ultimately reduces habitat suitability. It is estimated that at any one time approximately 16–17% of the forest area may contain suitable habitat for field voles (Lambin, Petty and MacKinnon, 2000).

Trapping regime

One permanent trapping grid, consisting of 100 Ugglan live multiple-capture traps in a 10 × 10 array, was established within each of 2 clear-cuts, identified as PLJ and KCS. Traps were spaced at 5 m intervals giving a grid size of 0·25 ha. Trapping was carried out every 4 weeks from March 2002 to March 2004 excluding the months of December and February for logistical reasons ($n = 23$ *primary sessions*). Traps were pre-baited for 3 nights to maximize capture rates, then set with crushed oats and carrot and checked 5 times (*secondary sessions*) at dawn and dusk over a 3-day period.

All field voles were tagged on their first capture occasion with a subcutaneous Passive Induced Transponder (PIT) tag (Labtrac by AVID plc, East Sussex, UK) thus giving each individual a unique 9-digit identification number. In addition, and on the first recapture occasion during each subsequent primary trapping session, we recorded each individual's sex, mass (to the nearest 0·5 g), coat colour (juvenile, adult/juvenile or adult) and location within the trapping grid and estimated their body condition (Cavanagh, 2001). Briefly, body condition is based on an index derived from palpating the fat deposits over the spine and hips. Scores for each ranged from 1 (low) to 5, giving a combined body condition index ranging between 2 and 10.

Individuals were assigned to 1 of 2 maturity classes on the following criteria. Mature adults: males weighing ⩾19 g with an adult coat and showing signs of sexual maturity (testes either abdominal-+ or scrotal); females weighing ⩾19 g with an adult coat and showing signs of sexual maturity (nipples medium or large, vulva perforate or plugged, pubic symphysis open or slightly open or presently gestating). Immature adults/juveniles: males weighing <19 g with a juvenile or adult/juvenile transitory coat and abdominal testes; females weighing <19 g with a juvenile or adult/juvenile transitory coat, closed pubic symphysis, small nipples and a non-perforate vulva.

All field voles were checked for ectoparasites on their first capture occasion each primary session by brushing or blowing against their fur while holding them in a gloved hand. However, as we neither wished to remove fleas, as happens when animals are combed over a water bath, nor overestimate infestation by wrongly counting moving fleas multiple times, it was considered inappropriate to attempt to name the exact number of fleas on any given individual. We therefore adopted an alternative method that more accurately reflected potential inprecision whereby we scored the combined prevalence of all flea species (Siphonaptera: *Ctenophthalmus nobilis, Peromyscopsylla silvatica spectabilis, Malaraeus penicilliger* and *Rhadinopsylla pentacantha*) on a semiquantitative scale of 1–3, with

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1 = 1–2 fleas, 2 = 3–5 fleas and 3 = > 5 fleas. The range of flea species found on field voles within Kielder Forest was established as part of a separate study involving biannual monitoring (March (n = 381) and September (n = 219)) at 27 sites over a large spatial scale, where animals were killed and fleas identified in the laboratory (S. Telfer, unpublished data). The larger and relatively slower moving mole fleas (*Hystrichopsylla talpae*) were counted individually but pooled with the above species when creating a binary present/absent flea covariate for trypanosome analysis (see below). The relative abundance of each species changed throughout the year, with *C. nobilis* the most prevalent in March (0·328), followed by *M. penicilliger* (0·084), *H. talpae* (0·039) *P. silvatica spectabilis* and *R. pentacantha* (0·005). In September, *P. silvatica spectabilis* was most abundant (0·239), followed by *H. talpae* (0·147), *C. nobilis* (0·142), and *M. penicilliger* (0·078), with no report of *R. pentacantha* during the autumn survey (S. Telfer, unpublished data). However, as it was not possible to determine the relative competencies of each species as a vector for *T. microti* it was assumed that all species covaried equally throughout the year.

Blood sampling and PCR

Blood samples of approximately 20–30 μl were collected from the tip of the tail on the first capture occasion each primary session. Blood samples were centrifuged to remove excess serum and DNA was extracted from the remaining pellets using the alkaline digest method (Bown *et al.* 2003).

Then 5 μl of the DNA extract was used as a template in a nested PCR that targeted a variable region of the trypanosome 18SrRNA gene. Each 50 μl reaction also contained 25 μl of 2× PCR Master Mix (Abgene, Surrey, UK), 18 μl of ddH_20 and 1 μl of of the following external primers: TRY927F 5′ GAAACAAGAAACACGGGAG, TRY927R 5′ CTACTGGGCAGCTTGGA, for 30 cycles of 94 °C 30 s; 55 °C 60 s; 72 °C 90 s (Noyes *et al.* 1999, 2000). The products from the first-stage reaction were diluted 1 : 10 and 2 μl of this was used as template for the second round using 1 μl of the internal primers: SSU561F 5′ TGGGATAACAAAGGAGCA, SSU561R 5′ CTGAGACTGTAACCTCAAAGC, 1 μl of ddH20, 45 μl of ReddyMix® PCR Master Mix (Abgene UK) and the cycling conditions described above. Then 12 μl of the second-round PCR product were loaded onto a 1·5% agarose gel stained with ethidium bromide and visualized under UV light. Samples producing a band of ~540 kb were considered positive.

PCR sensitivity, estimated by comparing the number of false negatives (single negatives within a complete sequence of positives) to the totals number of potential false negatives was 91·47%. Where it was not possible to retest suspected false positive results (i.e. single positives within a complete sequence of negatives), the individual in question was excluded from the analysis (n = 27). DNA extraction, primer preparation, first and second-stage PCR were all carried out in separate dedicated rooms to reduce the risk of contamination.

Population estimates

Field vole population density within each primary session was estimated using the closed population model M_{th} within Program CAPTURE (Otis *et al.* 1978). Model M_{th} assumes capture probabilities vary between secondary sessions and show heterogeneity amongst individuals (Pollock, 1982). Due to bad weather during the January 2003 primary session (#11), both grids were only trapped 3 times and density was estimated using the Minimum Number Alive method (Krebs, 1966). When extrapolating from density/grid to population density/ha we assumed an effective trapping area of 0·3 ha (55 m × 55 m), equal to an additional 0·5 trap-width on all sides, to allow for an edge effect.

Statistical analysis

Both the prevalence of fleas and trypanosome infection were modelled separately as binary response variables (fleas: present/absent, trypanosomes: positive/negative) using Generalized Linear Mixed Models (GLMM) with binomial errors and a logit link function (Paterson and Lello, 2003). In the absence of model selection criteria for GLMM that can distinguish between non-nested models, prior to the inclusion of random effects we investigated temporal patterns in flea and trypanosome prevalence, with the best seasonal GLM selected based on the Schwarz Criterion (SC) (Turchin, 2003). SC, otherwise known as the Bayesian Information Criterion, selects the most probable model based on fit and sample size with an additional penalty for complexity, and is similar in function to the unbiased Akaike Information Criterion (AICc) (Johnson and Omland, 2004). As with AICc, alternative models with differences in SC values <2 (ΔSC) are not considered to differ in their ability to describe the data (Burnham and Anderson, 2002). We investigated which of 8 seasonal variables best described temporal variation in flea and trypanosome prevalence. The seasonal variables ranged from a monthly pattern through several combinations with varying length of winter and summer periods: for example, bimonthly, starting in March 2002 (6 seasons), trimonthly, starting March 2002 (4 seasons), and in 4-month groupings starting April 2002 (3 seasons). We also considered 3 varying length 2-season models (winter-summer) and whether prevalence levels varied between years.

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Using the best seasonal model as a starting point, further analysis was conducted in a 2-stage process, with population level covariates investigated first and then individual level covariates. As all individuals within a grid experience the same density on any given trapping session, and multiple recordings were taken from many individuals over time, trapping session and individual were included as random effects using the GLIMMIX macro in SAS (Littell *et al*. 1996).

The population level covariates considered were year, site, present density and lag-densities of 2, 4 and 6 months and, for trypanosome prevalence, the proportion of hosts infested with fleas in the present session as well as at lags of 1–4 months. Two-way interactions between each of the continuous variables and year, site and season were also considered. Model selection was based on a step-down procedure, eliminating interactions first and retaining only those variables significant at the 2% level based on SAS type III *F*-test values (SAS/STAT, 1992).

Using the best model that incorporated seasonal and population level variables as a starting point individual covariates were then investigated in a similar manner. The individual level covariates considered were sex, maturity, whether the individual was new or recaptured from a previous session (N-R), 1 of 4 body mass based categories used as a proxy for age (<17 g; 17–24 g; 25–39 g and $\geqslant 40$ g) (Cavanagh *et al*. 2004) and a classification of the body condition index as follows: BC index $2–4=1$, BC index $5–6=2$, BC index $7–10=3$. For trypanosome prevalence we also included the semi-quantitative scale of flea prevalence as well as a binary measure representing whether fleas were present or absent regardless of the level of infestation. All 2-way interactions between seasonal, population and individual level variables were considered. Cross-correlation analysis between flea and trypanosome prevalence (see below) was carried out using SPSS V11.

RESULTS

Descriptive summary

A total of 1212 *M. agrestis* were used in the analysis, giving an effective sample size, including recapture occasions, of 3004. The structure of the population by sex and site is given in Table 1. The percentage of males was 59% at PLJ and 56% at KCS. The total number of individuals caught was similar between the 2 grids though recapture rates were higher at KCS. In addition, wood mice (*Apodemus sylvaticus*) were occasionally caught at both sites in low numbers (maximum 20 within any session), and bank voles (*Clethrionomys glareolus*), at similarly low numbers, at KCS only.

Table 1. Summary of the number of individuals caught by sex and site

	Sex	Site 1 (PLJ)	Site 2 (KCS)	Total
New captures	Male	362	336	698
	Female	252	262	514
	Total	614	598	1212
Recaptures		776	1016	1792
Total captures		1390	1614	**3004**

The two populations had similar prevalences of fleas and trypanosome infection (Fig. 1). Across all capture and recapture occasions, 57% of males (927/1627) and 42% of females (578/1377) were found to have some level of flea infestation and 39% (642/1627) of male blood samples and 36% (500/1377) of female blood samples were PCR positive for *T. microti*. The duration of trypanosome infection was variable, with 1 individual testing positive for 9 consecutive sessions and a further 2 individuals positive over 8 sessions. However, there was little evidence for the occurrence of secondary infections, with only 2 individuals testing positive again for 2 or more consecutive sessions following an initial period of recovery.

Peak density on KCS rose from $\sim$350/ha during late summer 2002 to 570/ha in August 2003 (Fig. 1A). A similar pattern was seen on PLJ, with densities rising from 210/ha in August 2002, to 717/ha in August 2003 (Fig. 1D). Lowest densities were recorded during June–July 2002 (36–73/ha) on PLJ and during April–June 2002 on KCS (73–86/ha). Flea prevalence on both grids increased during the early part of the year and remained relatively constant over much of the summer before returning to low levels over the winter months (Fig. 1B, E). Trypanosome prevalence appeared to increase rapidly, though later in the year than flea prevalence, then declined steadily to a low point during March and April (Fig. 1C, F).

Flea prevalence

The best-fit seasonal model for flea prevalence included 2 seasons: winter (November–March) and summer (April–October) (ΔSC >3 compared to next best model). The random terms individual and session both explained significant variation in the probability of flea infestation amongst hosts (Individual: $Z=2{\cdot}9$, $P=0{\cdot}0019$; Session: $Z=3{\cdot}58$, $P=0{\cdot}0002$) and were retained throughout the full modelling process. Of the population level parameters, the following were included in the final stage analysis: season ($F_{(1,1589)}=20{\cdot}20$, $P=<0{\cdot}0001$) density ($F_{(1,1589)}=6{\cdot}4$, $P=0{\cdot}0115$) and season$*$density ($F_{(1,1589)}=0{\cdot}0157$, $P=0{\cdot}0157$). The final

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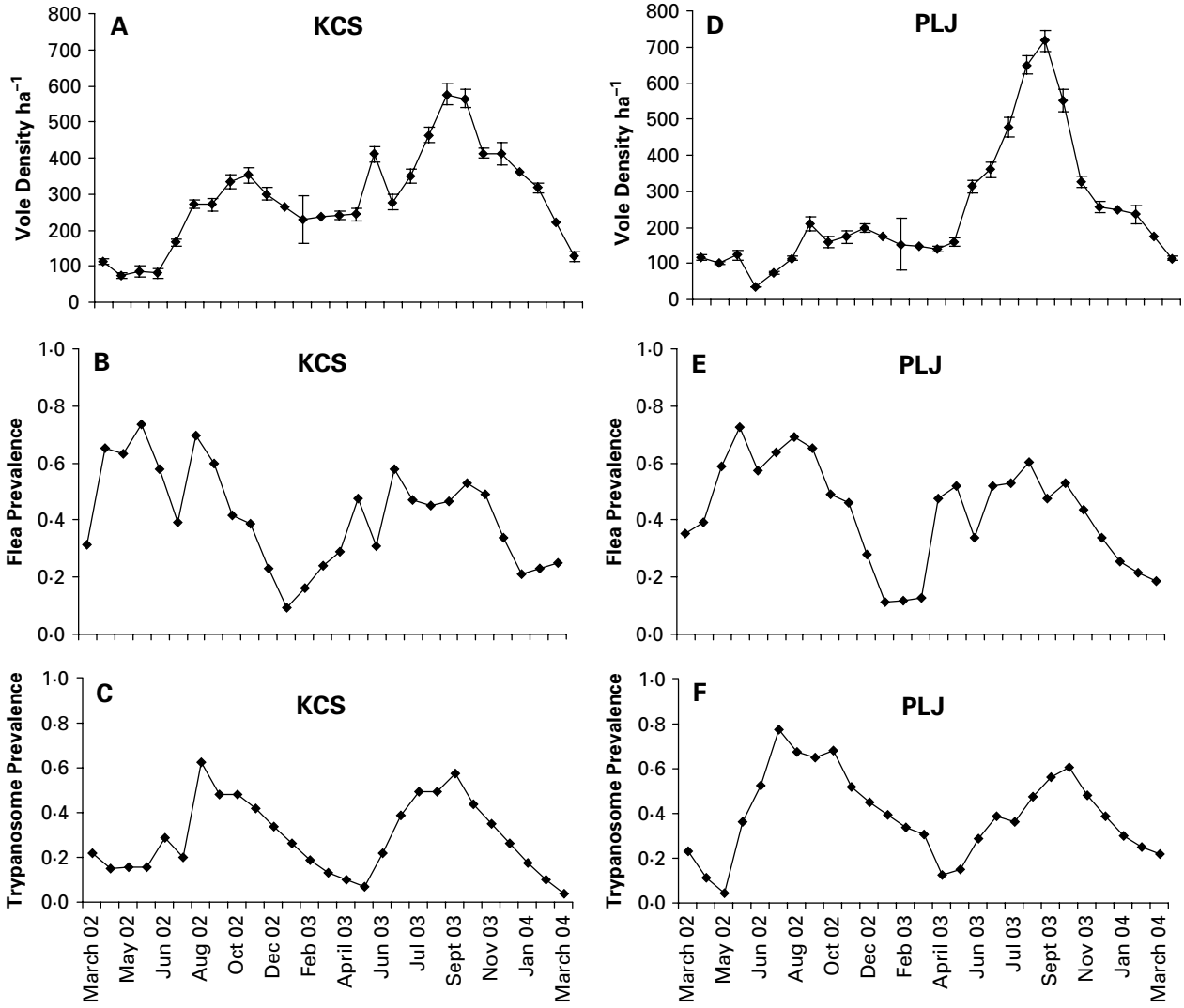

Fig. 1. Temporal variation in *Microtus agrestis* population density/ha (± S.E.) (A and D), flea prevalence (B and E) and trypanosome prevalence (C and F) on trapping grids KCS and PLJ respectively from March 2002 to March 2004.

model retained the interaction between season and density and also included the individual level factors mass ($F_{(3, 1589)} = 1{\cdot}09$, $P = 0{\cdot}3513$), sex ($F_{(1, 1589)} = 39{\cdot}98$, $P = < 0{\cdot}0001$) and density * mass ($F_{(3, 1589)} = 6{\cdot}03$, $P = 0{\cdot}0004$). Parameter estimates are given in Table 2. Overall, males are significantly more likely to be associated with fleas than females, and the absence of interactions between sex and other terms suggests the difference remains effectively constant over all ranges of density, mass and season and their interaction terms.

In general, the probability that an individual was infested with fleas increased with the current population density of field voles, with the relationship showing a steeper slope in the winter (density * season interaction, Table 2). The interaction term density * mass was driven mainly by significant differences between mass categories in the slopes relating the probability of infestation to density in the summer season. During the summer, larger animals (mass categories 3 and 4) were more likely to be infested with fleas at higher densities, whilst smaller animals (mass categories 1 and 2) showed no such relationship (Fig. 2).

Trypanosome prevalence

The best-fit model had 4 seasons: 'spring' (March–May) 'early summer' (June–July (encompassing 3 sessions)), 'late summer-autumn' (August–October) and 'winter' (November–January). The random terms individual and session contributed significant variation to the presence of trypanosome infection (Individual: $Z = 8{\cdot}8$, $P = < 0{\cdot}0001$; Session: $Z = 3{\cdot}97$, $P = < 0{\cdot}0001$) and were again retained throughout the analysis. After consideration of the population level parameters, the following were included in the final stage analysis: season ($F_{(3, 1589)} = 30{\cdot}24$, $P = < 0{\cdot}0001$) and flea prevalence at lag-3 months ($F_{(1, 1589)} = 11{\cdot}32$, $P = 0{\cdot}0008$). After consideration of individual level parameters an interaction between mass and whether the animal

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Table 2. Parameter estimates on a logit-scale for the final flea prevalence GLMM model

(Test statistics and associated *P* values for class variables are directed at the hypothesis that variation between class levels equals zero. For mass (4 levels), estimates and associated test statistics are directed at the hypothesis of zero variation between levels 1–3 in relation to level 4.)

Effect	β Estimate	S.E.	*t*	*P*
Intercept	0·07926	0·4631		—
Winter	−1·9011	0·4230	−4·49	<0·0001
Summer	0			
Density	0·006979	0·003617	1·93	0·0538
Mass 1 (<17 g)	0·8089	0·5323	1·52	0·1288
Mass 2 (17–24 g)	0·2401	0·4766	0·5	0·6145
Mass 3 (25–39 g)	0·2609	0·4639	0·56	0·5739
Mass 4 (>39 g)	0			
Female	−0·5246	0·08297	−6·32	<0·0001
Male	0			
Density∗*mass 1*	−0·01348	0·003896	−3·46	0·0006
Density∗*mass 2*	−0·00754	0·003614	−2·09	0·037
Density∗*mass 3*	−0·00412	0·003581	−1·15	0·2505
Density∗*mass 4*	0			
Density∗*Winter*	0·01307	0·005403	2·42	0·0157
Density∗*Summer*	0			

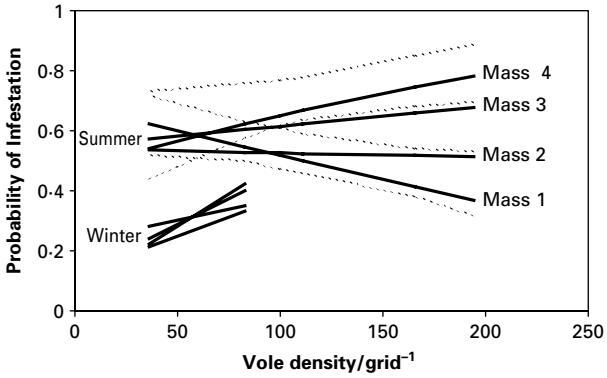

Fig. 2. Predicted probabilities of flea occurrence for males from all 4 mass classes (1 = smallest, 4 = largest). Plotted values are derived from reverse transformed linear predictors previously estimated from the model output in Table 2. Probabilities vary according to the interactions between mass ∗ density and season ∗ density but are shown here in terms of 'summer' and 'winter' over a realistic range of density estimates for the appropriate season. Estimates for females show a similar relationship but with approximately 11% lower overall values. Dotted lines represent 95% CI associated with estimates for mass groups 1 and 4.

Table 3. Parameter estimates on a logit scale for the final trypanosome GLMM model

(Test statistics and associated *P* values for class variables are directed at the hypothesis that variation between all other levels relative to the last mentioned level 4 equals zero.)

Effect	β Estimate	S.E.	*t*	*P*
Intercept	−2·5884	0·4337	—	—
Fleas lag3	1·9552	0·5810	3·36	0·0008
Mass 1 (<17 g)	1·7287	0·6258	2·76	0·0058
Mass 2 (17–24 g)	0·9667	0·2300	4·20	<0·0001
Mass 3 (25–39 g)	0·3730	0·2175	1·71	0·0866
Mass 4 (>39 g)	0	—	—	—
Spring	−0·6950	0·2882	−2·41	0·0160
Early Summer	0·9141	0·2787	3·28	0·0011
Summer-Autumn	1·4472	0·2487	5·82	<0·0001
Winter	0	—	—	—
New (N)	0·6612	0·4087	1·62	0·1059
Recapture (R)	0	—	—	—
Mass 1∗*N*	−3·0378	0·7289	−4·17	<0·0001
Mass 1∗*R*	0	—	—	—
Mass 2∗*N*	−1·1942	0·4313	−2·77	0·0057
Mass 2∗*R*	0	—	—	—
Mass 3∗*N*	−0·1177	0·4440	−0·27	0·7910
Mass 3∗*R*	0	—	—	—
Mass 4∗*N/R*	0	—	—	—
Mass 4∗*N/R*	0	—	—	—

was new or recaptured was also retained ($F_{(3, 1589)}$ = 13·64, *P* = <0·0001). Parameter estimates for the full model are given as logit values in Table 3.

The interaction between mass and N-R is driven by the fact that the recaptured animals in the lower 2 mass categories have higher probabilities of infection than new animals, whilst new animals in the upper 2 mass categories have higher probabilities of infection than recaptured animals (Fig. 3A). These patterns probably reflect differences between the true ages of new and recaptured animals classified within the same mass category. By definition, recaptured animals within mass category 1 are unlikely to be very young and may more closely resemble individuals within the second weight group in terms of actual age and exposure levels rather than small, new individuals. Similarly, new animals assigned to the upper mass categories may in fact be relatively young, compared to recaptured animals within the same mass category. A model with no mass ∗ new/recapture interaction, intended to demonstrate the convex shape of the age-prevalence distribution (Fig. 3B), suggests that the probability of infection is significantly lower among the youngest animals (<17 g), and reaches a peak within the middle two groups (17–25 g and 26–39 g) before declining again amongst the heaviest or oldest animals (>39 g).

The significant positive effect of flea prevalence at lag-3 months suggests an increase or decrease in flea prevalence at the population level is followed by a corresponding increase or decrease in the probability of trypanosome infection 3 months later. The probability of trypanosome infection is lowest during 'spring' (Fig. 4), which corresponds to the low levels of flea prevalence during the winter season (see Fig. 1B and E). Probability of infection then increases to 0·38 by 'early summer', then to 0·52 by 'late summer-autumn', again reflecting the increases in flea prevalence. By winter, the

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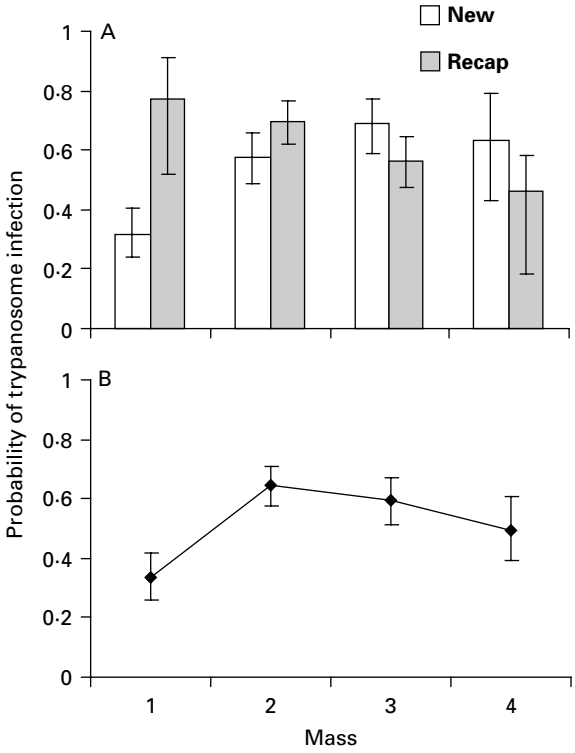

Fig. 3. (A) Probability of trypanosome infection for new and recaptured field voles within each mass group estimated during autumn (season 3), when infection prevalence is highest, as predicted by the best-fit model in Table 3. (B) An alternative model without the new/recapture * mass interaction to emphasize the convex shape of the age-prevalence distribution.

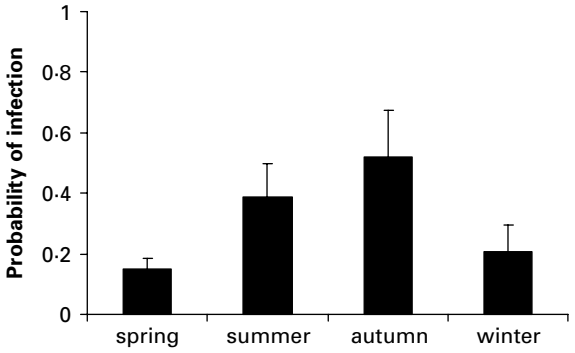

Fig. 4. Probability of trypanosome infection within each of the 4-seasonal categories predicted for new animals in mass class 2.

probability of infection has declined, which corresponds to the onset of the autumn reduction in flea prevalence.

Although the best-fit model selected flea prevalence at lag-3 months as a significant factor in determining current trypanosome prevalence, cross-correlation analysis suggests that the range over which this delayed density effect may be occurring actually ranges between lags 1–3 months (Fig. 5).

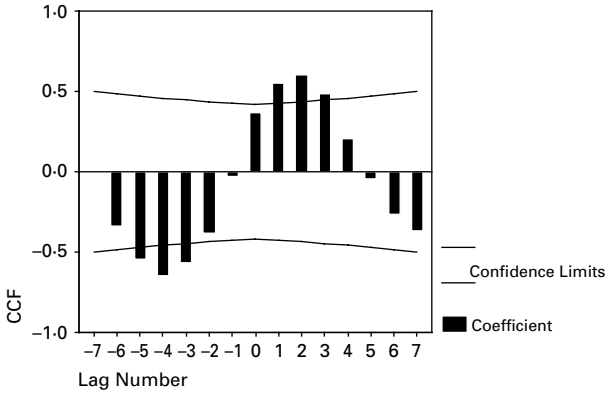

Fig. 5. Cross-correlation between flea prevalence and trypanosome prevalence up to lag-7. Positive coefficients suggest trypanosome prevalence was high 1–3 months following high flea levels. Negative values indicate low levels of trypanosome infection 3–5 months before high levels of flea prevalence. Confidence limits are set at 2 standard errors.

DISCUSSION

There was a high degree of heterogeneity within our field vole populations in their susceptibility to both ectoparasitism by fleas and infection by *T. microti*. Flea prevalence showed a strong seasonal pattern and was linked to field vole density, with males consistently more likely to be infested than females within the same age group. *T. microti* prevalence also showed a seasonal pattern and was related to flea prevalence with a lag of approximately 3 months, but showed no variation between host sexes.

Similar sex-differences in ectoparasite prevalence have been observed across a wide range of vertebrate taxa and are particularly common amongst mammals (Poulin, 1996). The underlying mechanisms behind such discrepancies are often divided into 2 broad categories, namely ecological and physiological (Zuk and McKean, 1996; Morales-Montor *et al.* 2004). The ecological mechanisms within our system are likely to be related to factors such as behavioural differences between the sexes in habitat use and hence variable exposure to parasites. Field voles are territorial during the breeding season and males aggressively defend relatively large non-contiguous home ranges, whereas females are less aggressive and often occupy smaller territories that overlap with those of neighbouring females (Reich *et al.* 1982; Taylor *et al.* 1984; Agrell Erlinge *et al.* 1996; Borowski, 2003). Young males are often forced to disperse as a result of aggressive encounters with older resident males within whose territory the natal nest is situated (Myllymaki, 1977). Fleas that increase fitness through dispersal would benefit through a greater association with male hosts (Lundqvist, 1988) – a suggestion apparently supported by data on the fleas of Californian ground squirrels, *Spermophilus beecheyi* (Bursten, Kimsey and Owings, 1997). Flea prevalence may thus be

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determined by host sex-specific differences in behaviour as well as behavioural modifications by fleas to adapt to those differences.

The physiological aspect of sex-bias in ectoparasite prevalence is commonly thought of as reflecting variation in the ability to mount an effective immune response. For example, increased levels of testosterone are known to depress both cell and humoral-mediated immune responses in reproductively active males, while stress-related corticosteroids are known to confound the production of antibodies (Khansari, Murgo and Faith, 1990). This effect is more pronounced in mammals that adopt a polygynous mating strategy due to the additional stresses associated with social (i.e. aggressive) interactions (Deerenberg *et al*. 1997; Klein and Nelson, 1999; Saino *et al*. 2000). Male *M. agrestis*, being highly polygynous, may encounter increasingly physiologically stressful situations during the breeding season when population density increases and territorial defence and competition for mates becomes intense (Pusenius and Viitala, 1993). Older males may therefore not only be physiologically more susceptible to flea infestation, but may also allow an ectoparasite to more readily obtain a longer bloodmeal and thus increase its fecundity levels (Lehmann, 1992; Tschirren, Fitze and Richner, 2003).

Older males supporting a disproportionate number of ectoparasites are an example of the commonly observed aggregated distribution of ectoparasites within host populations (Robbins and Faulkenberry, 1982; Shaw and Dobson, 1995). This has often been expressed as an 80/20 'rule', which suggests that, typically, 20% of the host population supports 80% of the parasite population (Woolhouse *et al*. 1997; see, for example, Perkins *et al*. 2003). However, while in the present case males in the upper two mass groups comprise 22% of the population, the number of fleas estimated to have been found on them is only approximately 36% of the total (where category 1 is taken as 1·5 fleas, category 2 as 4 fleas and category 3 as 8 fleas). This emphasizes that the 80/20 or any other 'rule' is inevitably an oversimplification, and that flea prevalence is determined by a range of factors including climate, season and host density as well as age and sex (Lang, 1996; Krasnov, Khokhlova and Shenbrot, 2002; Stark, 2002; Stanko *et al*. 2002).

Variation in flea prevalence between the upper and lower mass groups at high densities may be the result of a saturation effect whereby, due to a rapid increase of juveniles during the summer breeding season, there are relatively more small transient animals in the habitat which do not enter the burrow systems and hence do not come into contact with as many fleas as the older and larger territory holders. A similar saturation effect, which resulted in lower flea infestation rates on non-resident *Gerbillus dasyurus* was reported by Krasnov *et al*. (2002). It must also be noted that the scoring of flea prevalence based on the method described here (all flea species grouped) may hide species-specific patterns of infestation. Different flea species may be more or less associated with the host or the burrow system and may differ in their seasonal patterns (Stark, 2002).

In comparison to previous studies, the overall level of trypanosome prevalence here (59·6%) is relatively high. For example, Healing (1981) reported *Trypanosoma* prevalences of 10·3% in *M. agrestis* and 11·1% in *C. glareolus*; Turner (1986) found 20·2% in *C. glareolus* and only 2·2% in the wood mouse *Apodemus sylavaticus*; Bajer *et al*. (2001) found an average prevalence of 15% in *C. glareolus* over 3 years; and Pawelczyk *et al*. (2004) reported prevalence levels ranging from 20% to less than 10% in *M. arvalis* over 4 years. However, the differences between these and the present results may largely be due to differences in diagnostic methods. Traditionally, detection of trypanosomes in field samples has been based on microscopic observations of Giemsa-stained thin and thick blood smears or culture (Hoare, 1972; Noyes *et al*. 1999). The sensitivity of PCR-based methods is believed to be 2–3 times higher (Masiga *et al*. 1992; Desquesnes and Davila, 2002), which is borne out by the survey of Solano *et al*. (1999) of a cattle herd in Burkina Faso (microscopy 5·3%, PCR 11·5%), and by the study of De Almeida *et al*. (1998) on goats in The Gambia (microscopy 8%, PCR 24%). To our knowledge, ours is the first study to employ PCR-based methods in a longitudinal survey of trypanosome dynamics in a natural wildlife population.

The seasonal variation in *T. microti* prevalence fits well with recent observations by Bajer *et al*. (2001) and Pawelczyk *et al*. (2004) and corresponds, with a delay, with seasonal variations in flea prevalence. Trypanosome prevalence was positively related to the prevalence of fleas within the population during the preceding 3-month period. Although the regression model selected past flea prevalence at a lag of 3 months, the range over which the cross correlation coefficient between the two time series is significant includes the additional lags of 1 and 2 months. This is consistent with our understanding of *T. microti* infection, which remains detectable within the host by microscopy for $\geqslant$70 days (Hoare, 1972), and presumably for some time longer during the decline phase by PCR (Desquesnes and Davila, 2002). Due to the seasonal dynamics of fleas, flea prevalences will be temporally autocorrelated. The problem of distinguishing between correlated explanatory variables in multiple regression is likely to explain why only a lag at 3 months was selected in the GLMM analysis. However, the important point biologically is that the cross-correlation analysis and the GLMM both indicate that trypanosome prevalence is linked to past rather than current flea prevalence.

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The probability of trypanosome infection was lowest amongst individuals within the youngest age/weight class (<17 g) and highest amongst individuals in the second and third weight groups (17–24 g and 25–39 g), declining again in the heaviest, or oldest group, representing reproductively mature and overwintered animals. The convex or humped shape of the age-prevalence distribution, referred to as a Type III curve by Hudson and Dobson (1995), may be related to age-dependent changes in host susceptibility, such as behavioural adaptations related to vector avoidance, or an increase in infection-induced host mortality (Hudson and Dobson, 1995). However, considering the low pathogenicity of rodent trypanosomes in general (Hoare, 1972), and the fact that older field voles are more heavily parasitized by fleas and thus more exposed to infection than the younger animals, neither readily explains our observations. Alternatively, a convex age-prevalence curve may be generated when older individuals develop a degree of acquired immunity following infection, as has been observed in mice experimentally infected with *T. musculi* and *T. lewisi* (Albright and Albright 1991; Sato *et al*. 2004). The lowest levels of infection in the youngest cohort may be explained by their low probability of encountering fleas (see above), or possibly due to maternal antibodies acquired during development or through milk from the mother (Brambell, 1958).

The interaction term mass*new/recapture indicates that the probability of trypanosome infection amongst older individuals was higher in the 'new' category than those recaptured from a previous session. As recapture rates are high, this is likely to reflect a distinction between residents or territory holders (recaptured), and non-resident or transitory animals that have recently entered the trappable population (new). This suggests either that non-territory holders are more likely to become infected through differences in exposure levels, or that infected animals are less likely to become territory holders than their uninfected counterparts. However, the absence of a mass*new/recapture interaction in the flea prevalence model suggests that resident and transitory animals of similar ages are equally exposed to fleas and hence to the possibility of trypanosome infection. This in turn suggests a possible condition-dependent difference between new and recaptured individuals in their susceptibility to parasitaemia, which may lead to a subtle but potentially important effect of infection on host morbidity (Brown *et al*. 2000, 2003), exerting an influence on host dynamics that is only detectable at the population level.

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