

# Trypanosomes and haemosporidia in the buzzard (*Buteo buteo*) and sparrowhawk (*Accipiter nisus*): factors affecting the prevalence of parasites

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**Abstract** The prevalences of heteroxenous parasites are influenced by the interplay of three main actors: hosts, vectors, and the parasites themselves. We studied blood protists in the nesting populations of raptors in two different areas of the Czech Republic. Altogether, 788 nestlings and 258 adult Eurasian sparrowhawks (*Accipiter nisus*) and 321 nestlings and 86 adult common buzzards (*Buteo buteo*) were screened for parasites by the microscopic examination of blood smears and by cultivation. We examined the role of shared vectors and parasite phylogenetic relationships on the occurrence of parasites. In different years and hosts, trypanosome prevalence ranged between 1.9 and 87.2 %, that of *Leucocytozoon* between 1.9 and 100 %, and *Haemoproteus* between 0 and 72.7 %. Coinfections with *Leucocytozoon* and *Trypanosoma*, phylogenetically distant parasites but both transmitted by blackflies (Simuliidae), were more frequent than coinfections with *Leucocytozoon* and *Haemoproteus*, phylogenetically closely related parasites transmitted by different vectors (blackflies and biting midges (Ceratopogonidae), respectively). For example, 16.6 % buzzard nestlings were coinfecting with *Trypanosoma* and *Leucocytozoon*, while only 4.8 % with *Leucocytozoon* and *Haemoproteus* and 0.3 % with

*Trypanosoma* and *Haemoproteus*. Nestlings in the same nest tended to have the same infection status. Furthermore, prevalence increased with the age of nestlings and with Julian date, while brood size had only a weak negative/positive effect on prevalence at the individual/brood level. Prevalences in a particular avian host species also varied between study sites and years. All these factors should thus be considered while comparing prevalences from different studies, the impact of vectors being the most important. We conclude that phylogenetically unrelated parasites that share the same vectors tend to have similar distributions within the host populations of two different raptor species.

**Keywords** Avian blood parasites · Avian malaria · *Trypanosoma* · *Leucocytozoon* · *Haemoproteus*

## Introduction

Trypanosomatids and haemosporidians are relatively common parasites of birds. While haemosporidians (Haemospororida: *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*) are well studied due to their relatively simple diagnosis, avian trypanosomes (*Trypanosoma*; Kinetoplastea, Trypanosomatida) are rarely investigated, or inappropriate diagnostic methods are used, despite the fact that their prevalences are comparable to haemosporidia. Although haemosporidians (Alveolata) and trypanosomes (Excavata) are phylogenetically unrelated, their dioxenous life cycles share some similarities. These parasites multiply mainly in avian inner organs, whereas developmental stages predisposed for transmission to vectors are found in the peripheral blood. After ingestion by dipteran blood-sucking vectors, namely blackflies (Simuliidae), biting midges (Ceratopogonidae), mosquitoes (Culicidae), or hippoboscids (Hippoboscidae), parasites develop infective stages that

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are transmitted to a subsequent bird host during the next blood feeding.

In addition to parasite taxonomic bias, studies on blood parasites are also biased toward passerines, due to their simple trapping and handling. Studies on raptors (Falconiformes) have been mostly performed on migrating, captive, or injured birds (Krone et al. 2001, 2008; Valkiunas 2005; Sehgal et al. 2006; Outlaw and Ricklefs 2009), and generally less attention has been paid to breeding populations (Ashford et al. 1990, 1991). The studies of factors influencing the prevalences of trypanosomes and haemosporidians in the breeding populations of raptors have several advantages. Newly hatched nestlings are parasite free, and therefore susceptible to infection, parasites are acquired at the study site, the acute phase of infection has no effect on sampling since nestlings are immobile, and seasonal parasitemias are high (Valkiunas 2005).

Detailed studies aimed at the identification of avian trypanosome species are rather limited; most trypanosomes isolated from raptors to date belong to the *Trypanosoma avium* group, which is transmitted by blackflies (Diptera, Simuliidae; (Bennett 1961; Votýpka and Svobodová 2004)). More rarely, raptors can also harbor *Trypanosoma corvi*, which is transmitted by hippoboscids (Diptera, Hippoboscidae), and *Trypanosoma bennetti*, whose vector is unknown (Baker 1956a; Votýpka et al. 2004; Kirkpatrick et al. 1986; Zídková et al. 2012).

*Leucocytozoon* species are transmitted to raptors by blackflies. *Leucocytozoon toddi* is the only species that has been reported from raptors; however, these likely actually belong to at least two species: *Leucocytozoon mathisi* infecting sparrowhawks and *Leucocytozoon buteonis* infecting buzzards (Valkiunas et al. 2010). *Haemoproteus* (*Parahaemoproteus*) species are transmitted by biting midges (Diptera: Ceratopogonidae) (Valkiunas 2005; Martinsen et al. 2008). Five species of *Haemoproteus* have been reported from holarctic raptors, among which *Haemoproteus nisi* and *Haemoproteus buteonis* are widespread, *Haemoproteus elani* is rare, and two species occur in falconids only (Valkiunas 2005). Molecular typing reveals a higher diversity of avian haemosporidia. According to the MalAvi database, out of 29 different *Leucocytozoon* haplotypes from the order Falconiformes, two have been found in *Buteo buteo* and 4 in *Accipiter nisus* (plus 16 and 6 in other members of the genera). None of 11 *Haemoproteus* haplotypes detected in raptors (Falconiformes) originate from *B. buteo* or *A. nisus*, and just one from the genus *Buteo*.

The co-occurrence of parasites of different taxa within the same host raises the important question whether the prevalences are affected (i) by the vectors involved in parasite transmission or (ii) by the phylogenetic relationship of parasites. Furthermore, other factors such as nestling age, sampling date, number of siblings, home range of avian host, year, and locality may all influence parasite prevalences.

We expect that due to prolonged exposure, the prevalence of blood parasites in nestlings increases with age and that due to the seasonally increasing occurrence of (infected) vectors, later broods have a greater probability of infection. We also expect that bigger broods should have higher parasite prevalences, because the higher biomass increases the attractiveness for vectors. We evaluated whether these factors similarly influence the prevalences of blood parasites that are either phylogenetically related but transmitted by different vectors (*Haemoproteus* transmitted by ceratopogonids vs. *Leucocytozoon* transmitted by blackflies) or unrelated but transmitted by the same vectors (trypanosomes and *Leucocytozoon*, both transmitted by blackflies).

## Material and methods

### Statement of animal rights

This study was carried out in accordance with the Czech national law. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Faculty of Science, Charles University in Prague. Experiments were performed by licensed workers. Licensed ringers had permission to visit nests and to catch wild birds, including the sparrowhawk (specially protected species, Act N. 114/1992). Four of the authors (MS, LP, PV, PV) were licensed ringers during the study period, and at least one of them took part in each field sampling. The license was issued by the Ringing Centre of the National Museum in Prague.

### Sampling

Blood samples from raptors (125 µl) were taken from the brachial vein using a tuberculin syringe. Adult raptors were trapped close to their active nests using mist nets and a stuffed eagle owl (*Bubo bubo*) as a decoy. Nestlings were sampled directly at the nests. Authors MS and P. Volf had licenses to perform and control experiments on animals, issued by the Central Committee for Animal protection, at the time of the study.

### Study area and field work

The breeding populations of raptors and the prevalence of their blood parasites were studied in the following areas of the Czech Republic:

#### 1. Prague, Eurasian sparrowhawk (*A. nisus*)

An urban sparrowhawk population in the City of Prague (49.99–50.14 N, 14.30–14.62 E). The total area is ca. 500 km<sup>2</sup> and covers the area of the city and its suburbs. Habitats include

built-up areas, parks, gardens, and orchards as well as small tracks of woodland. Sparrowhawks breed in trees, usually in parks and gardens, across the whole area including the city center. The population size has been estimated at 90 to 120 breeding pairs (Peške 1990). The population was sampled during the breeding seasons 1996–2001 (mid-June to July). Ages of sparrowhawk nestlings were assessed according to the development of feathers (Peške 1987). The average age of sampled sparrowhawk nestlings was 22.3 days (SD=4.6, range 9–32,  $n=774$ ).

### 2. Prague suburbs, common buzzard (*B. buteo*)

A common buzzard population in the eastern suburbs of the City of Prague (49.94–50.00 N, 14.57–14.65 E). The total area is 60 km<sup>2</sup> and is characterized by extensive farmland with scattered small tracks of woodland, tree lines, and solitary trees, with dense human settlement and a dense network of roads. Buzzards breed in trees, usually in small tracks of woodland but also in tree lines along streams and sometimes also in solitary trees. The population size has been estimated at 15–20 breeding pairs (J. Matouš, pers. comm.) The population was sampled during the breeding seasons 2000–2002 (early May to June). Ages of common buzzard nestlings were estimated using measurements of the forearm or wing length (Voříšek and Lacina 1998). The average age of buzzard nestlings was 26.4 days (SD=5.3, range 16–35,  $n=61$ ).

### 3. South Moravia, common buzzard (*B. buteo*)

A common buzzard population in the forest complex “Milovický les” located in the Protected Landscape Area and Biosphere Reserve Pálava (48.48–48.51 N, 16.39–16.44 E). The study area is a 22-km<sup>2</sup> oak-hornbeam forest complex surrounded by intensively managed farmland. The population density of common buzzard is high in the area, with 32 to 51 breeding pairs confirmed in 1993 to 1995 (Voříšek 2000). Two game preserves for red and fallow deer (*Cervus elaphus*, *Dama dama*), and fallow deer and mouflon (*Ovis orientalis*), respectively, comprise the majority of the forest. Nearly 20 % of the total forest area is open habitats (e.g., clear cuts, fields). The population was sampled during the breeding seasons 1996–2001 (early May to June). The average age of buzzard nestlings was 24.1 days (SD=6.3, range 10–39,  $n=245$ ).

The active nests of both raptor species were searched for by a combination of systematic searches for nests (complete census) and observations of adults attending the nests with nest material or food. The nests were directly inspected, in most cases, several times during the breeding season. All nests found were marked on detailed maps allowing a determination of individual home ranges.

Since different raptor species have different habitat preferences, their populations were sampled in different habitats,

and any between-species comparison of prevalence would be misleading. Hence, we focus on variation in the prevalence within host species.

### Diagnostic methods

Blood samples from raptors were acquired, and trypanosome cultivation was done as described elsewhere (Votýpka et al. 2002). Briefly, 125  $\mu$ l of blood was taken from the brachial vein using a tuberculin syringe. Blood smears were air dried, fixed with methanol, and stained with Giemsa (Sigma). Microscopic examination was performed at  $\times 1000$  magnification for 10 min, which in our hands corresponds to approximately 30,000 erythrocytes. Additionally, slides were checked at  $\times 160$  magnification for 5 min, to find *Leucocytozoon* infections of low intensity. We have identified parasites to the generic level, since the splitting of the data to genetical lineages would decrease the statistical power of our analyses.

### Data analysis

As this is an exploratory study, we focused on detecting patterns in the data and estimating effect sizes, rather than on formal hypothesis testing. Raw prevalence was calculated as the proportion of positive sampling units, either individuals (nestlings, adults) or broods. A whole brood was classified as positive if  $\geq 1$  nestling in the brood was positive.

The frequency of coinfections with different parasite genera was evaluated by constructing a 2<sup>3</sup> contingency table (*Trypanosoma*  $\times$  *Leucocytozoon*  $\times$  *Haemoproteus*). The eight-cell frequencies (with an added constant of 0.5) represented the response variable in a Poisson regression model (GLM with log link and Poisson error distribution) fitted separately to each host category. We first fitted the full model to check for a three-way interaction (interpreted as triple associations among parasites). Next, we removed the three-way interaction and examined the reduced model with all two-way interactions (interpreted as double associations between parasites). All parameters were estimated by the maximum likelihood method implemented in Proc Genmod (SAS Institute, Inc. 2013).

The effects of multiple predictor variables on the infection status of individual nestlings or broods (coded 1=positive, 0=negative) were evaluated by fitting a logistic mixed model (GLMM with logit link and binomial error distribution) to each host-parasite combination. The prevalence of *Haemoproteus* in sparrowhawk nestlings was too low for analysis. We present regression coefficients (on logit scale) estimated by the full model containing all predictor variables that were a priori deemed important. The fixed effect predictors were brood size (1–6 nestlings), nestling age (9–37 days), sampling date entered as a Julian date (130–202), and

sampling locality (S. Moravia vs. Prague; analyzed only for buzzards). The random effects were year and brood identity (in models for individual nestlings) or year and home range identity (in models for whole broods). For random effects, we modeled random intercepts and assumed variance component variance-covariance structure. All parameters were estimated by the residual pseudo-likelihood method implemented in Proc Glimmix (SAS Institute, Inc. 2013). To visualize model predictions, we plotted prevalence against nestling age/Julian date while keeping the other variables fixed at a modal value (brood size) or at about the midpoint of the observed range of values (age/date).

## Results

### Prevalence of blood parasite genera

We detected three genera of blood parasites in the studied raptor populations: *Trypanosoma*, *Leucocytozoon*, and *Haemoproteus* (Table 1). *Plasmodium* and microfilariae were not found. Trypanosome infections were first detected in a 14-day-old sparrowhawk and 19-day-old buzzard; *Leucocytozoon* was first detected in a 16-day-old sparrowhawk and 17-day-old buzzard and *Haemoproteus* in a 23-day-old sparrowhawk and 22-day-old buzzard.

### Coinfections with different parasite genera

Combined infections were checked in individual nestlings, whole broods, and adults (Table 2). We did not find any triple association among all three parasite genera in any host category (Table 3). Of the three possible double parasite combinations, we found a consistent positive association between trypanosome and *Leucocytozoon* in all host categories except in adult sparrowhawks, where the effect was still positive, but weaker. A positive association between *Haemoproteus* and *Leucocytozoon* was less consistent (Table 3).

### Factors affecting prevalence in nestlings of buzzards and sparrowhawks

Brood size, nestling age, Julian date, year, and study locality were evaluated for their effects on *Trypanosoma*, *Leucocytozoon*, and *Haemoproteus* prevalences. The effects were evaluated for individual nestlings (Table 4) and whole broods (Table 5).

We found only weak, yet consistent, effects of brood size—prevalences at the level of individual nestlings tended to decrease with increasing brood size (Table 4), while prevalences at the level of whole broods tended to increase with brood size (Table 5). The prevalences of all parasite genera consistently increased with host age (Fig. 1) and with Julian date (Fig. 2) at both the nestling (Table 4) and brood (Table 5)

**Table 1** The prevalences of blood parasites in sparrowhawk and buzzard nestlings, broods, and adults, by localities and years

Host	Trypanosoma		Leucocytozoon		Haemoproteus	
	Nestlings	Adults	Nestlings	Adults	Nestlings	Adults
Sparrowhawk 1996	(0)	(0)	0.019 (52)	1.000 (20)	0.000 (52)	0.300 (20)
Sparrowhawk 1997	0.092 (152)	0.824 (51)	0.067 (180)	0.927 (55)	0.028 (180)	0.473 (55)
Sparrowhawk 1998	0.031 (129)	0.596 (47)	0.052 (135)	0.820 (50)	0.000 (135)	0.300 (50)
Sparrowhawk 1999	0.159 (170)	0.667 (54)	0.145 (173)	0.844 (64)	0.000 (173)	0.234 (64)
Sparrowhawk 2000	0.324 (145)	0.872 (47)	0.184 (152)	0.979 (48)	0.000 (152)	0.271 (48)
Sparrowhawk 2001	0.365 (96)	0.727 (22)	0.198 (96)	0.650 (20)	0.000 (96)	0.150 (20)
<i>Sparrowhawk</i>	<i>0.184 (692)</i>	<i>0.738 (221)</i>	<i>0.117 (788)</i>	<i>0.879 (257)</i>	<i>0.006 (788)</i>	<i>0.304 (257)</i>
Buzzard, Moravia 1996	(0)	(0)	0.778 (18)	(0)	0.000 (18)	(0)
Buzzard, Moravia 1997	0.019 (52)	0.636 (11)	0.224 (67)	0.818 (11)	0.015 (67)	0.727 (11)
Buzzard, Moravia 1998	0.021 (48)	0.667 (9)	0.316 (57)	0.750 (8)	0.105 (57)	0.875 (8)
Buzzard, Moravia 1999	0.125 (32)	0.800 (5)	0.471 (34)	1.000 (5)	0.000 (34)	0.800 (5)
Buzzard, Moravia 2000	0.036 (55)	0.667 (3)	0.236 (55)	1.000 (3)	0.327 (55)	1.000 (3)
Buzzard, Moravia 2001	0.270 (37)	1.000 (1)	0.571 (42)	1.000 (1)	0.333 (42)	1.000 (1)
<i>Buzzard, Moravia</i>	<i>0.080 (224)</i>	<i>0.690 (29)</i>	<i>0.366 (273)</i>	<i>0.857 (28)</i>	<i>0.143 (273)</i>	<i>0.821 (28)</i>
Buzzard, Prague 2000	0.632 (19)	(0)	0.737 (19)	(0)	0.105 (19)	(0)
Buzzard, Prague 2001	0.643 (42)	1.000 (1)	0.690 (42)	1.000 (1)	0.071 (42)	0.000 (1)
Buzzard, Prague 2002	0.607 (28)	(0)	0.690 (29)	(0)	0.000 (29)	(0)
<i>Buzzard, Prague</i>	<i>0.629 (89)</i>	<i>1.000 (1)</i>	<i>0.700 (90)</i>	<i>1.000 (1)</i>	<i>0.056 (90)</i>	<i>0.000 (1)</i>

Numbers of screened individuals are shown in brackets. Total numbers for each host by locality are in italics

**Table 2** Frequency of coinfections with different parasite combinations in sparrowhawk and buzzard

Parasite			Sparrowhawk			Buzzard		
T	L	H	Nestlings	Broods	Adults	Nestlings	Broods	Adults
0	0	0	0.759	0.576	0.056	0.463	0.299	0.000
1	0	0	0.110	0.173	0.069	0.042	0.044	0.000
0	1	0	0.053	0.068	0.144	0.204	0.263	0.036
1	1	0	0.071	0.168	0.412	0.166	0.190	0.179
0	0	1	0.003	0.010	0.000	0.048	0.051	0.143
1	0	1	0.000	0.000	0.019	0.003	0.007	0.000
0	1	1	0.001	0.000	0.056	0.048	0.095	0.143
1	1	1	0.003	0.005	0.245	0.026	0.051	0.500
Total number			692	191	216	313	137	28

A whole brood was classified as positive if  $\geq 1$  nestling in the brood was positive. Parasite combinations (table rows) represent exclusive categories; hence, the total prevalence for, e.g., trypanosome in sparrowhawk nestlings, is equal to  $0.110+0.071+0.003=0.184$ . The values of total prevalence calculated in this way may differ slightly from those shown in Table 1 because of different sample sizes

*T Trypanosoma*, *L Leucocytozoon*, *H Haemoproteus*; 1=positive, 0=negative

levels. Prevalences differed markedly between the two studied buzzard populations, *Haemoproteus* being more prevalent in S. Moravia, while *Leucocytozoon* and trypanosomes were more prevalent in Prague.

After accounting for the variation due to the above fixed effects, there was still a detectable component of variation in prevalences among the annual samples in some host-parasite combinations (Table 1) when the year was treated as a random effect (Tables 4 and 5). The consistent variation in prevalences among broods (except for *Haemoproteus*, possibly due to low prevalence; Table 4) indicates that the infection status of individual nestlings within a brood was correlated (i.e., not independent). On the contrary, we did not find clear support for a consistent variation in prevalences among individual home ranges (Table 5), suggesting that the infection status of individual broods within a home-range (across years) was not correlated.

## Discussion

### Prevalence of blood parasite genera

The prevalences of all three parasite genera (*Trypanosoma*, *Leucocytozoon*, and *Haemoproteus*) detected in the studied raptor populations were consistently higher in adults than in nestlings. This seems expectable, since the exposure to vectors increases with age. Moreover, some infections might remain undetected at the time of sampling, due to the prepatent period (the time from infection to the demonstration of the parasite in the blood). This will cause higher bias in nestling samples due to their low age, sometimes even comparable to the prepatent period. On the other hand, some infections in adults may be cleared, or there may be parasite-related mortality. The higher prevalence in adults might therefore suggest that the infections are lifelong, chronically patent, and no significant parasite-

**Table 3** Associations among different parasites in sparrowhawk and buzzard

Parasites	Sparrowhawk			Buzzard		
	Nestlings	Broods	Adults	Nestlings	Broods	Adults
T×L	<i>2.203±0.247</i>	<i>2.091±0.374</i>	0.651±0.401	<i>2.127±0.321</i>	<i>1.443±0.444</i>	<i>2.788±1.193</i>
T×H	0.260±0.880	-0.121±1.052	0.546±0.361	-0.312±0.421	-0.165±0.468	-0.459±1.039
L×H	<i>2.103±0.876</i>	0.793±1.054	<i>1.046±0.513</i>	<i>0.755±0.365</i>	0.618±0.458	-0.335±1.338
T×L×H	0.084±1.878	-0.636±2.283	1.572±1.584	0.437±1.014	0.512±1.118	-2.068±2.713

Analysis is based on a Poisson regression model applied to a three-way contingency table (Table 2: see for frequency of parasite combinations), separately for each host category. Regression coefficients ( $\pm$ SE) show relative strength and direction of associations in combined infections. Estimates for the triple association are from the full model, while estimates for the three double associations are from a model from which the triple association was removed. Formal statistical significance at  $p<0.05$  is indicated in italics

*H Haemoproteus*, *L Leucocytozoon*, *T Trypanosoma*



**Table 4** Logistic mixed models showing effects of predictor variables on prevalence in buzzard's and sparrowhawk's nestlings

Effect	Sparrowhawk		Buzzard		
	T	L	T	L	H
Fixed effects					
Brood size	-0.209±0.158	-0.145±0.184	-0.507±0.408	-0.138±0.259	0.345±0.493
Age	<i>0.097±0.039</i>	<i>0.176±0.047</i>	0.094±0.059	<i>0.137±0.035</i>	<i>0.172±0.068</i>
Julian date	<i>0.071±0.025</i>	<i>0.071±0.029</i>	0.025±0.046	0.011±0.025	<i>0.155±0.051</i>
Locality			-3.42±0.709	-1.354±0.567	2.937±1.325
Random effects					
Year	<i>0.769±0.659</i>	0.078±0.191	0.047±0.314	0.475±0.515	4.295±3.396
Brood	<i>1.628±0.413</i>	<i>2.433±0.524</i>	<i>2.027±0.75</i>	<i>0.825±0.38</i>	0.910±0.651
Individuals ( <i>n</i> )	680	771	277	325	325
Broods ( <i>n</i> )	188	212	123	145	145

The data unit is an individual nestling. Each host-parasite combination represents one separate model. Regression coefficients (±SE) show relative strength and direction of fixed effects. Effect of locality (buzzard only) is shown for S. Moravia vs. Prague (=reference category). For random effect, the estimated variance components (±SE) are shown. Formal statistical significance at  $p < 0.05$  is indicated in italics

*H Haemoproteus, L Leucocytozoon, T Trypanosoma*

related mortality occurs. However, the repeated sampling of adult sparrowhawks revealed that while *Trypanosoma* infections seem to be lifelong, *Haemoproteus* and *Leucocytozoon* infection status varies in both directions, although the overall prevalences in the studied population remains stable (Votýpka et al., unpublished observations; see also Synek et al. 2013a). The majority of numerous studies comparing young and adult hosts detected the higher prevalences of haemosporidians in adults (Valkiunas 2005). Studies on trypanosomes are scarce, and no differences between passerine age groups have been detected; however, trypanosomes have typically been

diagnosed on slides (Merino and Potti 1995; Deviche et al. 2010), and thus probably underestimated (Apanius 1991).

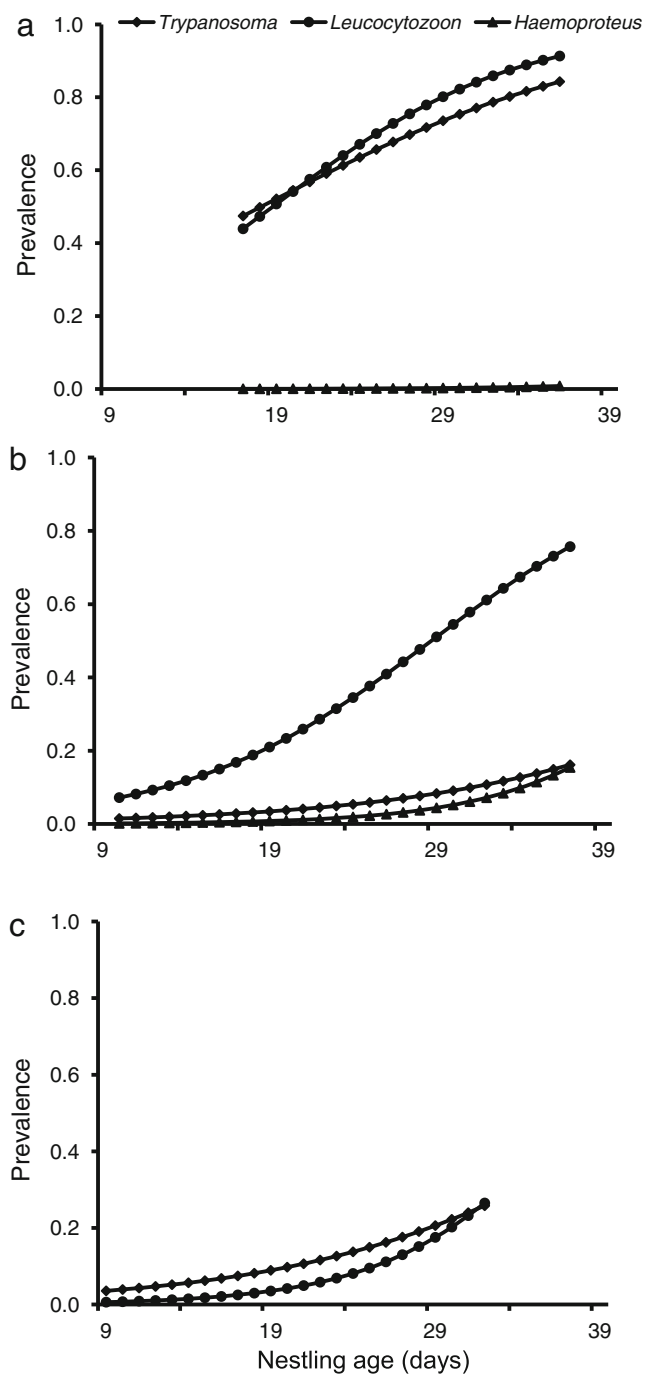
The prevalence of *Leucocytozoon* in nestlings was higher than that of *Haemoproteus*. While comparing the prevalences of different parasites, one has to bear in mind that the detectability of the infection is influenced, among other factors, by the prepatent period. The youngest sparrowhawks reported to be infected with *Leucocytozoon* were about 12 days old (Ashford et al. 1991). We detected *Leucocytozoon* in a 16-day-old sparrowhawk and a 17-day-old buzzard. While data on the prepatent period of *Haemoproteus* in raptors are

**Table 5** Logistic mixed models showing effects of predictor variables on prevalence in buzzard's and sparrowhawk's broods

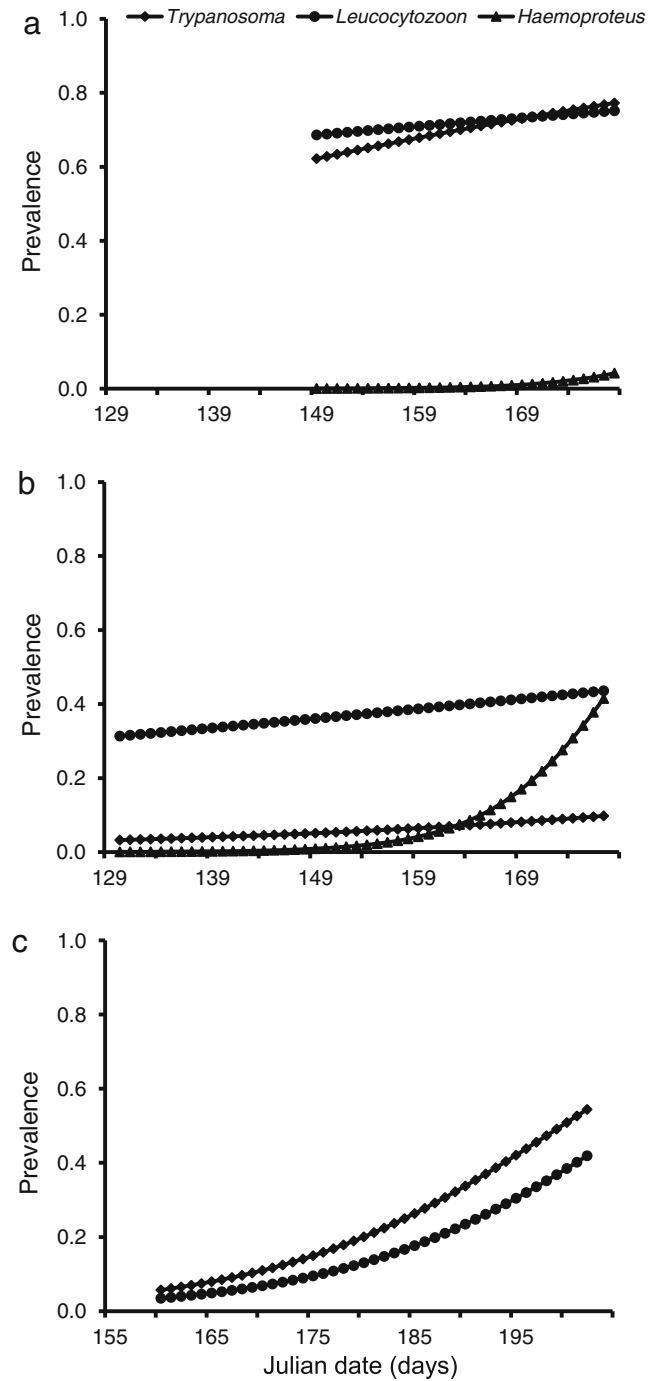
Effect	Sparrowhawk		Buzzard		
	T	L	T	L	H
Fixed effects					
Brood size	0.091±0.169	0.120±0.178	0.107±0.431	0.537±0.329	0.815±0.494
Age	<i>0.113±0.049</i>	<i>0.146±0.052</i>	0.133±0.077	<i>0.214±0.053</i>	0.109±0.078
Julian date	0.055±0.028	0.055±0.029	0.041±0.049	-0.001±0.029	<i>0.136±0.048</i>
Locality			-3.258±0.878	-0.756±0.784	2.922±1.356
Random effects					
Year	<i>0.649±0.614</i>	0.158±0.243	x	0.563±0.742	3.806±3.067
Home range	0.679±0.475	0.732±0.522	1.034±0.842	0.178±0.563	x
Broods ( <i>n</i> )	188	212	123	145	145
Home ranges ( <i>n</i> )	110	115	93	109	109

The data unit is a whole brood, classified as positive if ≥1 nestling in the brood was positive. Each host-parasite combination represents one separate model. Regression coefficients (±SE) show relative strength and direction of fixed effects. Effect of locality (buzzard only) is shown for S. Moravia vs. Prague (=reference category). For random effect, the estimated variance components (±SE; x=not estimable) are shown. Formal statistical significance at  $p < 0.05$  is indicated in italics

*H Haemoproteus, L Leucocytozoon, T Trypanosoma*



**Fig. 1** Age-dependent variation in parasite prevalence in raptor nestlings. Prevalence was modeled using logistic mixed models fitted separately for each host-parasite combination (see Table 4 for model parameter estimates). The data unit was an individual nestling. The fixed effect predictors were brood size, nestling age, Julian date, and locality (only in buzzard); the random effects were year and brood identity. The prevalence predicted by the models for an observed range of nestling ages is plotted, with brood size set to the modal value (2 and 4 nestlings in buzzard and sparrowhawk, respectively) and Julian date set to the midpoint of observed range of dates (June 4 in buzzard, June 24 in sparrowhawk), separately for both localities; **a** buzzard in Prague, **b** buzzard in South Moravia, and **c** sparrowhawk in Prague. The prevalence of *Haemoproteus* in sparrowhawk nestlings was too low for analysis (see Table 1 for overall prevalence values)



**Fig. 2** Seasonal variation in parasite prevalence in raptor nestlings. Prevalence was modeled using logistic mixed models fitted separately for each host-parasite combination (see Table 4 for model parameter estimates). The data unit was an individual nestling. The fixed effect predictors were brood size, nestling age, Julian date, and locality (only in buzzard); the random effects were year and brood identity. The prevalence predicted by the models for an observed range of Julian dates is plotted, with brood size set to the modal value (2 and 4 nestlings in buzzard and sparrowhawk, respectively) and nestling age set to the midpoint of observed range of ages (25 days in both host species), separately for both localities; **a** buzzard in Prague, **b** buzzard in South Moravia, and **c** sparrowhawk in Prague. The prevalence of *Haemoproteus* in sparrowhawk nestlings was too low for analysis (see Table 1 for overall prevalence values)

lacking, in other bird species, it ranges from 11 days to 3 weeks (Valkiunas 2005; Atkinson 2008). For *Haemoproteus*, the youngest birds in which we detected infection were a 22-day-old buzzard and 23-day-old sparrowhawk. Although sparrowhawks were sampled later in the season, the host age at sampling was similar for buzzards and sparrowhawks. Interestingly, in both avian hosts, *Haemoproteus* is detected at a more advanced age than that of *Leucocytozoon*, suggesting that the prepatent period is longer for *Haemoproteus* than *Leucocytozoon* species in raptors.

The youngest bird with a trypanosome infection detected in this study was a 14-day-old sparrowhawk and a 19-day-old buzzard. The prepatent period of avian trypanosomes is possibly only 18–24 h, at least for *T. corvi* (Baker 1956b). However, buzzards were sampled earlier in the season, and their more advanced age at first detection may relate to the seasonal availability of infected vectors. The development of infective forms in vectors is affected by temperature, lasting about 5 days at 24 °C and longer at lower temperatures (Bennett 1961).

We used microscopy to diagnose haemosporidia infections. It has been shown that microscopy is just as sensitive as PCR diagnosis (e.g., Krone et al. 2008); moreover, PCR underestimates mixed infections (Valkiunas et al. 2006) and can give false-positive results due to the detection of sporozoites in the blood that may not represent specific parasites (Valkiunas et al. 2009). Generally, we do not expect any significant bias in the results due to the diagnostic methods used.

We have identified the parasites to the generic level, since identification into a level of species or strain was not required given the purpose of our study. Our main aim was to show that phylogenetically unrelated parasites that share the same vectors (*Trypanosoma* and *Leucocytozoon*) show similar prevalence patterns while closely related *Haemoproteus* and *Leucocytozoon* (which have different vectors) have different prevalences. Splitting of the data set would also decrease the statistical power of our analyses to detect patterns in prevalences.

#### Coinfection with different parasite genera

As expected, there was a strong positive association between *Trypanosoma* and *Leucocytozoon* in both nestlings and whole broods of buzzards and sparrowhawks and in adult buzzards. We explain this pattern by their transmission by the same vectors. Most raptors (over 90 %) in our studied populations were infected with trypanosomes of the “*T. avium*” group (Zídková et al. 2012), which are transmitted by blackflies of the subgenus *Eusimulium* (Votýpka et al. 2002; Votýpka and Svobodová 2004; Zídková et al. 2012). Haemosporidian sporozoites were repeatedly found in blackflies attacking raptor nestlings sampled in this study (Svobodová et al.,

unpublished). Since the majority of *Leucocytozoon* species are transmitted by blackflies, while *Haemoproteus* (*Parahaemoproteus*) is transmitted by biting midges, we suppose that these were *Leucocytozoon* sporozoites. Moreover, *Leucocytozoon* was detected by PCR exclusively in blackflies while *Haemoproteus* in biting midges (Synek et al. 2013b; Bobeva et al. 2014). This, together with the proven occurrence of midges and blackflies in the nests of these studied raptors (Votýpka et al. 2002; Votýpka and Svobodová 2004), supports the respective roles of these vectors in the transmission of haemosporidians.

The lack of a positive association between trypanosomes and *Leucocytozoon* in adult sparrowhawks is unexpected but might be caused by a higher diversity of infecting trypanosomes in adults. We have isolated a trypanosome strain belonging to the *T. bennetti* clade (Zídková et al. 2012), a species with an unknown vector. Moreover, predation has been suggested as a mode of transmission of avian trypanosomes in sparrowhawks (Dirie et al. 1990). Alternatively, a patent infection with *Leucocytozoon* might affect the probability of catching adult sparrowhawks, while in nestlings, there is not such a bias (Valkiunas 2005). Moreover, the repeated sampling of adult sparrowhawks has revealed that some *Leucocytozoon* infections are lost, while trypanosome infection seems to be lifelong (Votýpka et al., unpublished; see above).

*Haemoproteus* and *Leucocytozoon* infections in nestlings (but not in broods) were also positively associated, yet the evidence is generally weaker than those for the association between *Trypanosoma* and *Leucocytozoon*. This association of parasites transmitted by different vectors might be a by-product of the age of nestlings at sampling, in concordance with the low overall prevalence of *Haemoproteus*.

Wiehn et al. (1999) failed to find a significant dependence of coinfections between *Haemoproteus*, *Leucocytozoon*, and trypanosomes in adult Eurasian kestrels (*Falco tinnunculus*). However, the prevalence of *Leucocytozoon* in that study was very low (0–1 %). On the other hand, Kirkpatrick and Lauer (1985) found sharp-shinned hawks (*Accipiter striatus*) infected with trypanosomes to be more likely infected with *Leucocytozoon*, but not with *Haemoproteus*, and they suggested this is due to a shared vector.

In the white-winged crossbill (*Loxia leucoptera*), a positive association of trypanosome infection was found both with *Leucocytozoon* and *Haemoproteus* (Deviche et al. 2010). If we suppose the same vector groups as in raptors, this might be explained by the age of the birds as a cofactor of infection, since the study was done on adults. Then, if the trypanosome infection is lifelong as in the case of sparrowhawks, the observed pattern is a function of age.



## Factors influencing prevalences in nestlings

Two opposing mechanisms may have influenced the relationship between prevalence and brood size. First, prevalences at the level of individual nestlings tended to decrease with increasing brood size (Table 4), which is to be expected if more nestlings in a brood cause a dilution of vectors (Ratti et al. 2006). Second, prevalences at the level of entire broods tended to increase with brood size (Table 5), which is to be expected if nests with more nestlings have higher biomass, and are supposed to be more attractive for vectors. For instance, blackflies and midges are known to be more abundant in nests with more flycatcher (*Ficedula hypoleuca*) and tit (*Parus* spp.) nestlings (Martinez-de la Puente et al. 2009a, b). The probability of infection of an entire brood with *Leucocytozoon* was not shown to increase with increasing sparrowhawk brood size (Ashford et al. 1991). Although none of the brood size effects was formally statistically significant in our case, the change from a negative effect at the individual level (Table 4) to a positive effect at the brood level (Table 5) was consistent (the effect for *Haemoproteus* in buzzard was positive in both cases, but its slope increased) and in accordance with the above explanation.

The prevalences of all parasites increased with the age of nestlings/broods (Tables 4 and 5, Fig. 1). Prolonged exposure to vectors is thus positively correlated with prevalence. This suggests that there is no parasite-related mortality or that parasite-related mortality is overcompensated by new infections.

The prevalence of all parasites generally increased with date (Tables 4 and 5), being the most pronounced for *Haemoproteus* in buzzard. Since the increase of prevalence due to nestling age was statistically controlled for, the effect of date may be explained by the increasing occurrence of infected vectors throughout the season. Blackfly and midge abundance increases along with delayed hatching date in flycatchers and tits in Southern Europe (Martinez-de la Puente et al. 2009a, b; Tomas et al. 2008). In Central Europe, ornithophilic *Culicoides* species usually culminate in June (Chvála et al. 1980; Votýpka, unpublished); however, detailed data on ornithophilic species are lacking. The strong seasonal increase in the prevalence of *Haemoproteus* could be also affected by the low overall prevalence of this parasite in the population.

The duration of this study was not sufficient to evaluate long-term trends in prevalences. Nevertheless, variation in prevalence among annual samples (year treated as random effect) suggests caution in the comparison of results obtained in different years without taking this variation into account. Similarly, the markedly different prevalences of all three parasite genera in two buzzard populations demonstrate that prevalences may vary considerably among sampling locations within host species. On a smaller spatial scale, within a host population, we found little support for the effect of home

range on infection probability. This is likely due to the change of nest location in subsequent years within a home range (obligately for sparrowhawk, commonly for buzzard) (Hudec and Šťastný 2013). It seems that nest detectability is greatly influenced by its fine-scale location within the home range, which is the most important factor in visually orientating vectors such as blackflies.

## Clustering of infection in broods

As expected, infection with all three genera of blood parasites was not independent for nestlings in the same nest, i.e., a sibling of an infected nestling has a greater probability of being infected with the same parasite genus, and vice versa. Similarly, Ashford et al. (1991) found that broods tend to be either entirely uninfected or almost entirely infected with *Leucocytozoon*. This suggests that some nests are either easier to locate for vectors or, once a vector finds a nest, it may move between siblings while feeding, thus increasing the number of exposed individuals. Alternatively, the offspring of certain parents may have similar genetic predisposition for being infected, which differs between broods. Since it is unlikely that genetic predisposition for infection with haemoproteids and trypanosomes is similar due to their phylogenetic distance and consequently different physiology, we suppose that different exposure to vectors is the cause of infection clustering.

## Conclusion and future directions

Our data on the prevalences of blood parasites in nestling and adult raptors revealed that phylogenetically unrelated parasites that share the same vectors tend to have similar distributions within host populations. This emphasizes the importance of future vector studies for our knowledge of wildlife parasites. Such studies are recently under way for passerine trypanosomes and their vectors.

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