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Parasite pressure and its effects on blood parameters in a stable and dense population of the endangered Lesser grey shrike

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Abstract. The risk that pathogens and parasites pose to endangered species is increasingly evident. Nonetheless, this is frequently overlooked when considering causes of decline of species and conservation practices. Here, we study the ecto and haemoparasites of adult and nestling Lesser grey shrikes *Lanius minor* from a dense and stable breeding population in central Europe and their effect on host blood parameters. We found three species of haemoparasites (*Haemoproteus* sp., microfilariae tentatively assigned to the family Splendidofilariae and *Trypanosoma* sp.) and two ectoparasite taxa (*Menacanthus camelinus* and feather mites – Acarina-). Our data suggest that the studied population, located in an area with traditional and extensive farming, is not under a strong parasite pressure. Despite this, indirect measures of immunocompetence (haematocrit and sedimentation rate) showed an association between haemoparasites and health status: while haematocrit did not differ between parasitised and non-parasitised individuals, adult shrikes with haematozoa had significantly lower sedimentation rates than did non-parasitised birds.

Introduction

Existing and emerging pathogens pose unusual challenges for conservation measures because of their potential to change the numerical abundance and genetic composition of wild host populations (Altizer et al. 2003). There is increasing evidence that implicates pathogens and parasites in population declines (e.g. Cooper 1989; Daszak et al. 2003) or identifies them as important threats to the conservation of endangered species (see, for instance, Font 2003; Cuaron et al. 2004). Moreover, the emergence and spread of new diseases, either due to anthropogenic activity (through habitat fragmentation, climate shifts, environmental pollution…), or with no overt human involvement, is becoming increasingly evident (e.g. Daszak et al. 2000; Altizer et al. 2003). For instance, Weissenböck et al. (2002) have recently documented the emergence of the Usutu virus in Central Europe, probably introduced from Africa, to the Austrian bird population by migrating birds. Therefore, the evaluation of the impact of parasites and other disease agents on bird populations as well as

the importance of birds (mainly migrant species) as disease transmitters is increasingly needed (May 1988; Cooper 1989; Loye and Carroll 1995).

Most of the species of the Family Laniidae (shrikes) are in decline on a worldwide basis (Yosef 1994). This is the case of the Lesser grey shrike *Lanius minor*, a long-distance migratory passerine, wintering in southern Africa. Until the early 20th century, this species was a common breeder all over Europe but a decrease in its breeding range in the 1960s has led to the disappearance of the species from many western European countries. In recent decades, the range has even begun contracting in eastern Europe (Lefranc and Worfolk 1997) where the largest populations can be found (Tucker and Heath 1994). As with other shrike species, climatic changes and habitat deterioration due to changes in agricultural practice have been regarded as the main causes for its decline (Lefranc 1995, 1997). Nonetheless, the effect of parasites and other disease agents on shrikes has been scarcely documented (Lefranc and Worfolk 1997), the available information being mainly records of parasite occurrence and taxonomic works (e.g. Bennett et al. 1990; Skoracki et al. 2001, 2002; Votýpka et al. 2003). For the Lesser grey shrike, knowledge of parasites is particularly fragmentary and almost anecdotal except for helminths (Hromada et al. 2000).

The Lesser grey shrike, and many other shrikes, frequently inhabit landscapes shaped by extensive farming (Lefranc and Worfolk 1997). This ecosystem is particularly subject to changes that may affect parasites as a result of the changing host–vector habitat relationships (Bennett et al. 1982a; Merino et al. 1997). This is even more the case in eastern European countries that are rapidly changing to western-like production models that often imply drastic modifications to the rural landscape.

Here we present data on prevalence of ectoparasites and haematozoa in adult and nestling Lesser grey shrikes from one of the last stable and dense populations of this species on the current northwestern border of its breeding distribution (central Slovakia) (Krištín et al. 2000). We also study the effect of parasitism on host health on the basis of indirect measurements of immunocompetence and discuss the potential threat of parasites on the conservation of this species.

Methods

During 1999, data on parasites of Lesser grey shrikes were collected from a population breeding in a 20 km^2 plot in Central Slovakia (40 °35′–38′ N, 19 °18′–22′ E, 450–850 m above sea level), on the southern slopes of the Polana Mountains Biosphere Reserve. This area supports one of the last stable and dense populations of the Lesser grey shrike in Central Europe (Krištín et al. 2000).

Thirty-four adult shrikes were clapnetted during the breeding season (mid May until the end of June). We also sampled 28 nestlings from 7 nests during June and July 1999. Each individual was ringed, measured, weighed and

scanned for ectoparasites before being released. Ectoparasites were searched by two persons for about 10 min. Mallophaga were scanned by carefully examining the bird's head, throat, belly and rump. Acarina were sought by extending and exposing against the sun the wing and tail feathers.

Blood samples were taken from the basilic vein and thin blood smears were prepared, air-dried, fixed with absolute ethanol and stained with Giemsa stain. A 200× lens was used to look for extracellular parasites in one half of each smear. Intracellular stages of haematoza were sought at 400× but, in contrast to the usual method (see, for instance, Merino and Potti 1995), we scanned the whole smear following the recommendations of Cooper and Anwar (2001). The oil immersion objective was used when a possible parasite was located at 400×. Numbers of intraerythrocytic parasites were estimated by counting the number of parasites per 2000 erythrocytes (Godfrey et al. 1987). Numbers of extraerythrocytic parasites were estimated by counting the number of parasites per 100 fields.

To measure sedimentation rate, the capillary tube containing blood was positioned vertically for 4 h at 4 °C. The length of plasma and blood cells was measured with an electronic calliper to the nearest 0.01 mm. The sedimentation rate (proportion of blood sedimented per 4 h) was calculated as the distance occupied by plasma divided by the total length of the blood column (plasma + cells). Capillaries were centrifuged at 4000 rpm for 10 min immediately afterwards to calculate the haematocrit value (packed cell volume).

Since sedimentation rate depends on haematocrit, we removed the effect of the latter by means of ANCOVAs, as recommended by García-Berthou (2001).

Samples infected with *Haemoproteus* sp., *Trypanosoma* sp. and microfilariae were deposited in the collection of the Museo Nacional de Ciencias Naturales (Madrid, Spain) (reference numbers MNCN 35.02/23, MNCN 33.03/6 and MNCN 11.02/382, respectively). Feather mite samples were lost during mailing, and therefore we have no information about the species. Nonetheless, we prefer to report their occurrence.

Results

Blood parasite prevalence

We identified two species of blood parasites in the blood smears of 34 adult birds: apicomplexan *Haemoproteus* sp. and microfilariae (Subfamily Splendidofilarinae, tentatively belonging to the genus *Splendidofilaria*). The prevalence of *Haemoproteus* was 17.7% (6 out of 34 adult birds infected). The range of intensity of infection was 0.4 parasites/2000 erythrocytes to 90 parasites/2000 erythrocytes. Of the 34 adults, 4 (11.8%) were infected with microfilariae, the parasite load ranging $< 1/100$ fields to 20/100 fields.

In total, 8 out of 34 adults were infected with at least one haematozoa parasite. Two adult shrikes were infected with both haematozoa parasites.

Date of sampling did not differ between birds parasitised and non-parasitised by haematozoa (Mann–Whitney U test, $z = 0.82$, $p = 0.41$, $n = 8, 25$).

None of the 28 nestlings was infected with *Haemoproteus* or microfilaria but we found one nestling infected with a trypanosome *Trypanosoma* sp.

Ectoparasite prevalence

We recorded the occurrence of *Menacanthus camelinus* (Menoponidae, Mallophaga) in six adults (17.6%) and the occurrence of feather mites (Acarina, probably *Syringophilopsis kristini*) in four adults (11.8%). Parasitation by ectoparasites seemed to be more frequent as the season advanced because birds holding ectoparasites were trapped later than non-parasitised birds (median date of capture for parasitised and non-parasitised birds: 9th June and 27th May, respectively, Mann–Whitney U test, $z = 2.31$, $p = 0.02$, $n = 9, 23$).

No ectoparasite was found on nestlings.

Mixed infection by both ecto and endoparasites was registered in five cases: one adult was infected with three parasite taxa (*Haemoproteus*, Acarina and microfilaria) and four adults were infected by two taxa (microfilaria and *M. camelinus*, *M. camelinus* and feather mites, *Haemoproteus* and *M. camelinus, Haemoproteus* and microfilaria).

Parasitism and health

We found no differences in haematocrit between birds infected with haematozoa and non-infected birds (Mann–Whitney U test, $z = 0.41$, $p = 0.68$, $n = 6, 21$) (Figure 1). In contrast, birds infected with haematozoa had a significantly lower sedimentation rate than did non-infected individuals (ANCOVA, $F_{1,24} = 12.76$, $p = 0.015$, covariate: $F_{1,24} = 5.55$, $p = 0.027$) (Figure 1).

Neither the haematocrit nor the sedimentation rate differed in birds with and without ectoparasites (haematocrit: Mann–Whitney U test, $z = 0.25$, $p = 0.80$, $n = 5, 22$; sedimentation rate: ANCOVA, $F_{1,24} = 0.32$, $p = 0.57$, covariate: $F_{1,24} = 3.56$, $p = 0.071$).

Discussion

The decline of shrikes in Europe and North America has generally been ascribed to climatic fluctuations and habitat deterioration (Lefranc and Worfolk 1997, but see Herremans 1997). Nevertheless, the impact of parasites and other disease agents on shrikes has been overlooked (Lefranc and Worfolk 1997). Our data suggest that the studied population (located in an area with traditional and extensive farming and limited habitat degradation) is not under

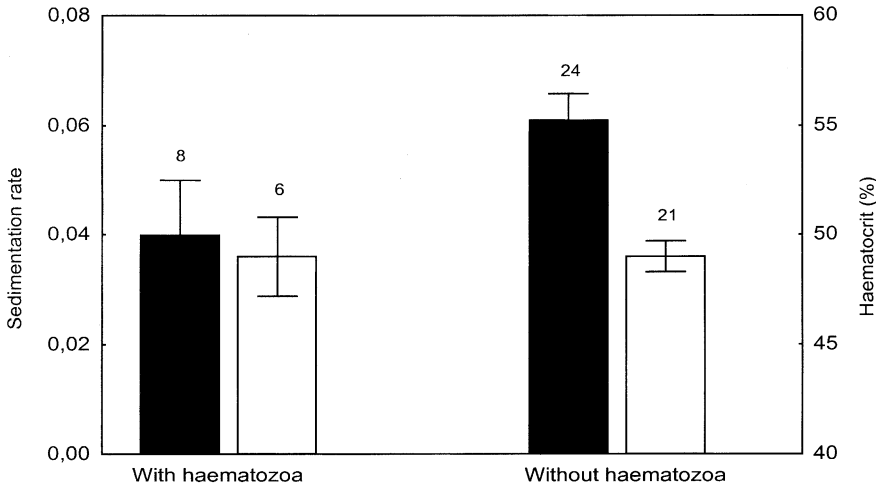

Figure 1. Sedimentation rate (filled bars) and haematocrit (empty bars) of adult Lesser grey shrikes with and without haematozoan parasites (*Haemoproteus* sp. and/or microfilaria). Means and standard errors are shown. Figures on the top of the bars represent the sample size.

a strong parasite pressure. At least nine species of haematozoa (see Bennett et al. 1982b; Bishop and Bennett 1992; Shurulinkov and Golemansky 2003), 24 species of helminths (Hromada et al. 2000) and 7 species of ectoparasites (Clay and Hopkins 1951; Lunkaschu 1970; Price 1977; Krištofík et al. 2002; Skoracki et al. 2002) have been described in the Lesser grey shrike. We only registered three haematozoa and two ectoparasites. Moreover, prevalences were relatively low. For comparison, Votýpka et al. (2003) found for a related species (red backed shrike *Lanius collurio*) that ca. 73% of adults were parasitised with *Haemoproteus lanii*. Likewise, Krištofík et al. (2002) reported that the mite assemblages in nests of Lesser grey shrike (most of them collected from the same population here studied) were poorer in number of species and abundance of specialised haematophagous parasites of birds in comparison to other passerines.

There is a growing evidence that parasites have an effect on a great variety of host fitness components (Rätti et al. 1993). Votýpka et al. (2003) found that *H. lanii* infections influenced reproduction and sexual characters of red-backed shrikes. We did not find any effect of parasitism on haematocrit, which suggests that infection did not cause anaemia (see, for instance, Ots and Horak 1998 for similar results). In contrast, adult Lesser grey shrikes parasitised by haematozoa had a lower sedimentation rate than did uninfected birds. A similar result was reported by Szép and Moller (2000), that found a higher sedimentation rate in nests of sand martins *Riparia riparia*, where ticks *Ixodes lividus* were removed in comparison with parasitised nestlings. In general, the sedimentation rate is increased in all acute, general infections (NCCLS 2000) but many factors may influence this parameter. For instance, the occurrence of

intraerythrocytic parasites may increase cell volume and decrease sedimentation. Szép and Moller (2000) pointed out that sedimentation rate is an indirect estimate of protein content in the plasma, with immunoglobulins constituting an important component (Sharma et al. 1984) and thus, a high sedimentation rate reflects superior health status.

Ectoparasites can play a role in modulating the relationship between condition and immune defence (Blanco et al. 2001). We found no effect of ectoparasites on haematocrit or sedimentation rate. Nonetheless, O'Brien et al. (2001) showed that nestlings parasitised by blowfly (*Calliphora*) larvae showed no reduction in haematocrit levels, but a significant reduction in haemoglobin values.

Agroecosystems are fragile environments where dramatic changes are liable to happen. Such changes may have an effect on parasites as a result of the changing host–vector habitat relationships (Bennett et al. 1982a). Our finding of a mild parasite pressure in an area with traditional and extensive farming agrees with the low prevalences of haematozoa in birds sampled from habitats with extensive farming (Tella et al. 1996; Blanco et al. 1997). Merila et al. (1995) showed that greenfinches (*Carduelis chloris*) had high prevalences of haematozoa in relatively unaltered habitats in Spain and Scandinavia, while birds from central Europe and other areas located near large human populations had low prevalences of haematozoa. In contrast, Bennett et al. (1991) did not find differences in prevalence of blood parasites after comparing data obtained in the same area with a lapse of 50 years, despite the change to a more agricultural environment.

Despite the mild parasite pressure found in this study, we could detect an association between haemoparasites and health status. The importance of other parasites, such as helminths (see Hromada et al. 2000), should be assessed to ascertain their influence on this endangered species. We believe that to understand the decline of the shrikes, and of many other inhabitants of agricultural habitats, it is neccessary to include parasitic species in ecological and environmental studies and to collect more information on the health and condition of individuals to confirm the suitability of habitats for birds. Our study provides information on parasites of a stable and dense population of Lesser grey shrikes breeding in a suitable environment due to the maintenance of traditional, extensive farming. This information may prove useful in the short term, given that dramatic changes are likely to occur on a large scale in agroecosystems of eastern Europe.

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