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STANDARD SAMPLING TECHNIQUES UNDERESTIMATE PREVALENCE OF AVIAN HEMATOZOA IN WILLOW PTARMIGAN (*LAGOPUS LAGOPUS*)

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ABSTRACT: A total of 68 willow ptarmigan (*Lagopus lagopus* L.) was collected during September 1995 from two localities in Troms County, northern Norway. Thin blood smears were prepared and examined for blood parasites. Of the 68 willow ptarmigan examined, 94% harbored one or more species of hematozoa. There were four (6%), 44 (65%), 16 (24%), and four (6%) birds infected by zero, one, two, and three species of parasites, respectively. Prevalences at the coastal locality, Kattfjord (n=43), were *Leucocytozoon lovati* 86%, *Trypanosoma avium* (26%), and microfilariae (30%). At the inland locality, Iselvdalen (n=25), prevalences were *L. lovati* 96%, *T. avium* 12%, and microfilariae 0%. We also searched connective tissues for the filaroid nematode *Splendidofilaria papillocerca*; in Kattfjord this parasite only occurred in adult hosts where prevalence was 94%, but the parasite was not found in Iselvdalen. To estimate the efficiency of parasite detection by standard blood sampling techniques, we sampled peripheral blood from the brachial wing vein and blood from the pulmonary system from willow ptarmigan. Sampling peripheral blood from the brachial vein led to underestimates of the prevalence of microfilariae. There was no significant difference between *L. lovati* and *T. avium* prevalence in blood collected from the brachial vein or deep circulation. Age of host had a strong impact on prevalence, especially for *S. papillocerca* and microfilariae.

Key words: Hematozoa, *Lagopus lagopus, Leucocytozoon,* microfilariae, *Splendidofilaria, Trypanosoma,* willow ptarmigan.

INTRODUCTION

Most populations of birds are infected with blood parasites belonging to one or more genera among hemosporidians, trypanosomes, or filaroid nematodes (Atkinson and Van Riper, 1991). Such parasites have a potential for affecting mortality, reproduction, or other traits related to host fitness, and have been hypothesized to be important selective factors in birds (Loye and Zuk, 1991; Høglund et al., 1992; Redpath et al., 2000). However, empirical evidence that blood parasites have negative fitness effects are conflicting (Atkinson and Van Riper, 1991; Dale et al., 1996; Merino et al., 2000; Sanz et al., 2001). These studies have tried to correlate parasite intensities or prevalences to fitness related parameters, which stresses the need for reliable sampling techniques.

The majority of studies follow the same general sampling procedures; i.e., samples are prepared as thin blood smears from the peripheral circulation and blood is most often obtained by puncturing the brachial vein of birds or by clipping the tip of a claw. This may be an adequate sampling method for parasites which invade blood cells flowing passively in the blood stream and, therefore, should be more or less evenly distributed in host blood. However, parasites such as microfilaria that tend to congregate in deep circulation, especially in lungs where the flow rate of blood is slow (Robinson, 1954; Hawking, 1975), might be difficult to detect in samples of peripheral blood.

Trypanosomes might also congregate in deep circulation and typically exhibit chronic infection patterns with variable intensities (Barry and Turner, 1991; Dobson and Hudson, 1995). Bennett (1962) found that blood smears were inadequate for the detection of trypanosomes and microfilariae and recommended use of a hematocrit centrifuge technique for adequate detection of these parasites. Techniques for cul-

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turing trypanosomes have been described by Kirkpatrick and Lauer (1985), and have been shown to be more effective in detecting trypanosome infections than blood smears (Kirkpatrick and Suthers, 1988). However, in spite of all its shortcomings, the preparation of blood smears still remains the most used sampling technique, not only for hemosporidians, but also for trypanosomes (Redpath et al., 2000; Sanz et al., 2001) and microfilariae (Høglund et al., 1992; Forbes et al., 1994).

If parasites tend to congregate in deep circulation, parasite detection in standard blood smear preparations could be improved in postmortem studies if blood is sampled from the pulmonary system. In this study we used willow ptarmigan (*Lagopus lagopus*) to investigate whether sampling of blood from deep circulation performed better for blood parasite detection than standard sampling of peripheral blood from the brachial vein.

MATERIALS AND METHODS

Forty-three willow ptarmigan were sampled during the hunting season (15–22 September, 1995) from a coastal locality on the island Kvaløya (69°40′N, 18°15′E; Troms County, northern Norway). Blood samples also were collected from 25 willow ptarmigan on 10–11 September, 1995 from an inland locality in Troms County, Iselvdalen (68°55′N, 18°50′E). Birds were classified as juveniles (2–3 mo old) or adults (≥14 mo) from moulting sequence and pigmentation of the primaries (Myrberget, 1975). Two blood smears were made from the brachial vein immediately after the birds were shot, following the techniques outlined by Bennett (1970). In addition, a blood smear was made from deep circulation by using a 2 ml syringe with a short needle (14 mm) attached. The needle was inserted pointing forwards along the dorsal side of the bird, making a small angle with the ribs of the host in order to obtain blood primarily from the lungs.

The esophagus, trachea, crop plus connective tissue, and fat deposits surrounding these organs were searched, using a stereomicroscope, for adult filaroid nematodes *Splendidofilaria papillocerca* (Lubimov, 1946). This nematode produces microfilariae found in blood smears of willow ptarmigan (Sonin and Barus, 1981). Blood smears were air dried and fixed in absolute methanol for 1 min, within 12 hr after collection. They were stained in phosphate buffered Giemsa (pH 7.2) for 10 min and examined microscopically. Fields were scanned for parasites starting from the beginning of the blood film, counting field by field towards the end of the film (see Godfrey et al., 1987). Blood smears were examined for 10–15 min at low magnification (400×) followed by scanning of at least 100 fields at high magnification (1,000×). For this study, only the prevalence of each parasite species was recorded.

All terms describing parasite populations follow the definitions of Bush et al. (1997). All tests were two-tailed, and P-values ≤ 0.05 were considered significant. Yates corrected Chi-square was used to test differences in prevalences between age groups and different sampling methods. Fisher exact test was used to test for differences in infections between the two localities. The Mann-Whitney U-test was applied for testing differences in parasite intensities.

RESULTS

Of the 68 willow ptarmigan examined, 94% harbored one or more species of blood parasites. There were four (6%), 44 (65%), 16 (24%), and four (6%) birds infected by zero, one, two, and three species of parasites, respectively. Adult *S. papillocerca* were found in connective tissues, while *L. lovati*, *T. avium*, and microfilariae were found in blood smears (Table 1).

Prevalences varied with age (Table 1). Mature *S. papillocerca* and their microfilariae were exclusively found in adult hosts. *Trypanosoma avium* was found in both age classes but prevalence was significantly higher in adult birds in Kattfjord (Table 1). *Leucocytozoon lovati* was common in adults and juveniles collected from both localities but no age-related difference in prevalence was detected.

Prevalence of microfilariae was significantly higher in smears of blood collected from deep circulation than in samples taken from the brachial vein (Table 2) for birds shot in Kattfjord. In all cases where microfilariae were found in at least one of the smears prepared from the brachial vein, blood samples from deep circulation were also positive. We did not observe microfilariae or *S. papillocerca* in birds shot in Iselvdalen.

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TABLE 1. Prevalences of *Splendidofilaria papillocerca* and blood parasites in blood smears from willow ptarmigan in Kattfjord and Iselvdalen (Troms County, Norway) September 1995. Prevalences are calculated as a total from peripheral blood and blood collected from deep circulation. Mean intensities of *S. papillocerca* are given in parentheses.

	Iselvdalen				Kattfjord			
	All birds $n=25$	Juveniles $n=11$	Adults $n=14$	P	All birds $n=43$	Juveniles $n=27$	Adults $n=16$	P
S. papillocerca	0.0	0.0	0.0	—	35 (4.0)	0.0 —	94 (4.0)	0.000
Microfilariae	0.0	0.0	0.0	—	30	0.0	81	0.000
Leucocytozoon lovati	96	100	93	NS[a]	86	89	81	NS
Trypanosoma avium	12	9	14	NS	26	19	38	0.004

[a] NS=$P>0.05$.

Prevalence of *T. avium* tended to be higher in samples of blood from deep circulation (Table 2), but this result was not significant. Three samples of peripheral blood were positive for *T. avium* when the corresponding samples from deep circulation taken from the same three hosts were not.

For *L. lovati* no significant differences between blood taken from peripheral or deep circulation were found, and there was no difference in prevalence between coast and inland localities (Fisher exact, $P=0.24$). However, among 61 birds positive for *L. lovati*, infection was only detected in one of three blood smears taken from five birds. In the remaining 56 birds positive for *L. lovati,* the infection was detected in all three blood smears prepared from each individual host. This equals a 95% probability of successful detection of *L. lovati* if single blood smears had been used.

DISCUSSION

Samples of blood from the pulmonary tissues yielded prevalences for microfilariae that were significantly higher than samples collected from the brachial vein. Thus, sampling of blood from deep circulation improved detection rate of microfilaria. Use of blood from pulmonary tissues did not produce a significantly higher detection rate for *T. avium* and in three cases trypanosomes were found in one of two brachial vein samples taken from each host, but were undetected in samples from deep circulation. Increasing the number of blood smears from each host thus seemed to be more important than sampling location for improving detection rates for trypanosomes if microcentrifugation for microfilariae (Bennett, 1962) or blood cultivation for trypanosomes (Kirkpatrick and Lauer, 1985) cannot be employed. No differences in prevalence of *L. lovati* were found between standard sampling of pe-

TABLE 2. Prevalences of blood parasites in blood smears collected from the brachial vein (BV) and pulmonary circulation (PC) of willow ptarmigan collected in Kattfjord ($n=43$) and Iselvdalen ($n=25$), Troms County, Norway) 1995.

	Kattfjord			Iselvdalen		
	BV	PC	P	BV	PC	P
Leucocytozoon lovati	84	81	NS[a]	84	92	NS
Trypanosoma avium	9	19	NS	4	8	NS
Microfilariae	9	30	0.0004	0	0	—

[a] NS=$P > 0.5$.

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ripheral blood and blood from deep circulation.

Prevalence of *S. papillocerca* in adult hosts from Kattfjord was 94% in 1994, while prevalence of microfilariae in brachial vein samples from the same hosts was only 24% (Holmstad and Skorping, 1998). In 1995 there was a significant reduction in mean intensity of adult *S. papillocerca* in adult birds, compared to the intensity in the same area in 1994 ($Z=-2.41$, $P=0.02$). Despite of this reduction in adult filariid intensity, prevalence of microfilariae in samples of blood from deep circulation was 82% among adult hosts in 1995. Thus the prevalence of microfilariae probably was underestimated in 1994, when standard sampling of blood from the brachial vein was employed.

Microfilariae were not found in any juvenile bird. In a study conducted in the area in 1994, no microfilariae were found in juvenile birds despite a high prevalence of *S. papillocerca* (68%) in host tissues (Holmstad and Skorping, 1998). This probably indicates that *S. papillocerca* has a long prepatent period, which is consistent with previous studies of willow ptarmigan (Haaland, 1928) and other tetraonids (Borg, 1953). More surprising was the lack of *S. papillocerca* among juveniles sampled in this area in 1995 (Holmstad and Skorping, 1998). Moreover, we did not find filariid infection in birds at the inland locality in 1995, although prevalence was 16% in single smears of blood taken from the brachial vein at this locality in 1992 (unpubl. results). Although the reason for differences between years in transmission success remains unknown, our results suggest local blood parasite communities might undergo substantial year to year variation.

Infections of *L. lovati* were common at both localities. Hemosporidian parasites normally enter a chronic stage in autumn, when vectors are inactive or absent, which makes it difficult to detect parasites in blood smears (Atkinson and Van Riper, 1991; Valkiunas, 1998; but see Fedynich and Rhodes, 1995). In spite of this, prevalences of *L. lovati* recorded in willow ptarmigan in this study were high compared to other studies of this host-parasite system (Haaland, 1928; Bennett, 1972). The only study with comparable prevalences of *L. lovati* was conducted in summer when transmission occurs (Mahrt, 1981). Species of *Leucocytozoon* are characterized by long patency with a few parasites present in peripheral blood in autumn and winter (Valkiunas, 1998; unpubl. data). However, even the high prevalence of infection recorded in this study does not exclude sampling biases that could have lead to underestimates of prevalence. In most cases *L. lovati* was found in all of the three blood smears prepared from each bird, but in five cases only one of the three blood smears were positive for *L. lovati.*

If previous studies of blood parasites have suffered from underestimation of hematozoan prevalences as our results suggest, and some studies may have been conducted in the wrong place or wrong year, using possibly biased sampling methods (Valkiunas, 1998), evaluation of the effects of blood parasites would be difficult. This may explain conflicting results in the literature on this question. Thus, conclusions in any short-term field study in which measurable, negative fitness effects of hematozoa are sought will be dependent on choice of location, timing, and use of reliable sampling techniques.

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