Hematozoa in Thin Blood Smears

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LETTER TO THE EDITOR . . .

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We have read with interest several recent papers published in the Journal of Wildlife Diseases and elsewhere on avian hematozoa in thin blood smears. We are specifically concerned with the sampling and quantification procedures used in some of these studies, particularly in view of earlier concerns raised by Godfrey et al. (1987).

Workers have examined blood samples collected from both live-trapped and shot birds in several recent studies (Stacey et al., 1990; Bennett et al., 1991a; Mahrt et al., 1991; Telford et al., 1992; Garvin et al., 1993; O'Dell and Robbins, 1994). We could find no account as to whether these two very different sampling procedures were tested for possible inherent variation in parasitemia prior to pooling and interpretation of the data. Hopefully, this aspect was considered by the authors and inadvertently omitted in their papers. Using a mixture of sampling methodologies without regard to inherent variation resulting from different techniques seems precarious. First, it assumes that hematozoans are distributed similarly in the blood of both living and dead birds. Second, blood often is sampled at a different location in a live host than in a dead host. It does not seem likely that blood obtained via the brachial or tarsal veins in living birds (sampling peripherally circulating blood) and from the heart or deep core blood in dead birds represents uniformity in sample collection. We believe that such lack of sampling standardization represents poor experimental design. Data collected using different sampling methodologies should be tested to determine if pooling of the data is appropriate.

The use of hunter-shot birds (Castle and Christensen, 1990; Stacey et al., 1990; O'Dell and Robbins, 1994) sampled at hunter check stations represents a potential source of variation. Time periods between host death and blood collection can be extremely variable between host individuals sampled in this manner. We have seen birds presented to biologists at check stations that ranged from those recently killed (within 15 min) to those that had been dead for at least 3 hr. Sampling blood from these hosts assumes that parasite behavior is similar across the entire range of time periods (between host death and sample collection) in which hosts are sampled. Unfortunately, factors associated with hematozoan response to host death have not been assessed adequately for this assumption to be made unequivocally. We are aware of only one study (West and Starr, 1940) that anecdotally reported how varying time periods between host death and sample collection influenced detection of *Leucocytozoon smithi* in domestic turkeys (*Meleagridis gallopavo*). We believe that until there are rigorous studies that can resolve this issue, blood smears taken from dead birds should be made within a standardized time period as soon as possible after host death, and beyond which blood samples are not collected.

Several workers have presented only prevalence data in studies on hematozoa (Stacey et al., 1990; Bennett et al., 1991a, b; Mahrt et al., 1991; Telford et al., 1992; Forbes et al., 1994; Taft et al., 1994). It is uncertain as to what significance can be attached to presence and absence data on hematozoans from blood smears. Our view is based on the following observations. First, absence of hematozoans in blood smears does not necessarily indicate that the host is not infected. Host immune response and host-parasite mechanisms associated with prepatent, latent, and relapse periods are important factors that influence the prevalence and density of hematozoans found in circulating blood cells of the host. These

concepts are reviewed by Atkinson (1991) and Greiner (1991). Thus, low hematozoan density coupled with short examination times of blood smears could result in substantial misclassifications, such as, false negatives. Forrester et al. (1974) clearly demonstrated how wild turkeys initially classified negative for *Plasmodium* sp., based on blood smears, were positive when subinoculation techniques were employed. Second, ecological relationships between hosts and their parasites cannot be assessed adequately using only prevalence data. Even when density data are available, it is not always possible to determine periods of peak transmission potential or the relation of infection to disease. Certainly, conclusions regarding these parameters within host populations based on prevalence data alone are unfounded. Thus, without intensity data, the ecological and clinical significance of hematozoan infection remains obscure.

In reviewing those papers in which intensity (number of parasites/number of erythrocytes within infected hosts) was reported, we found that intensity often was calculated by direct counting of parasites and estimating the numbers of erythrocytes examined, based on the number of fields of view examined (Apanius and Kirkpatrick, 1988; Castle et al., 1988; Allan and Mahrt, 1989; Castle and Christensen, 1990). We believe that the estimated number of erythrocytes examined, derived from extrapolations, is inappropriate for the following reasons. First, due to the extreme variation of erythrocyte densities encountered on blood smears, estimated total numbers of erythrocytes examined, based on a subsample directly counted in several randomly chosen fields of view, will not provide reproducible results. Second, when intensity data are derived from partially quantified density data (actual number of parasites counted/estimated number of erythrocytes examined in each host individual), an estimation error is generated from the original estimated quantities (density values of each individual host). Statistical analyses of data using derivatives of actual and estimated quantities seems tenuous. Thus, we question the usefulness of presenting partially quantified intensity data that cannot be readily subjected to statistical analyses.

We have shown that quantification of *Haemoproteus maccallumi* (=*H. columbae* n. comb.) in blood smears from mourning doves (*Zenaida macroura*) must be made by directly counting a sufficient number of erythrocytes to provide an accurate estimate of the number of parasites/number of erythrocytes (Godfrey et al., 1987). Application of this methodology was used to evaluate intraerythrocytic hematozoan communities in hosts with high (Godfrey et al., 1990) and low (Fedynich et al., 1993) parasite densities. We realize that direct counts of a sufficient number of erythrocytes in some hosts can be tedious, due to low densities of parasites; it often requires several hours to count 10,000 erythrocytes. However, direct counts of both parasites and erythrocytes provide a reproducible estimate of parasite intensity that can be compared statistically using various intrinsic and extrinsic factors. Thus, until a better methodology is developed, we suggest that researchers directly count the number of erythrocytes when quantifying intraerythrocytic hematozoans.

Unfortunately, little effort has been directed toward developing quantification procedures for hematozoans such as *Leucocytozoon* spp. that may occur in both erythrocytes and leucocytes. Based on some data from wild turkeys, *L. smithi* density may vary concordantly with erythrocyte densities on blood smears (A. M. Fedynich, unpubl.; O. E. Rhodes, Jr., pers. comm.).

We believe that in view of these ideas, many researchers should upgrade and revise their experimental design. It is imperative that papers examining hematozoans clearly state how hosts were sampled, test for sampling bias if differing collection methods were used, include both prevalence and intensity data for intraerythrocytic hematozoans, and completely

describe how prevalence and intensity data were determined. Incorporation of these suggestions will provide readers with a better understanding of how the study was conducted and provide more interpretable, meaningful, and reproducible results.

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