Trypanosomes of Some Fennoscandian Birds

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Linear measurements and derived indices of trypanosomes from four species of Fennoscandian birds were compared to those reported for Trypanosoma avium, T. everetti, T. ontarioensis and T. paddae. The trypanosomes encountered in the Fennoscandian birds were identified as T. avium from Tengmalm’s owl Aegolius funereus and the pied flycatcher Ficedula hypoleuca, T. everetti from the great tit Parus major and collared flycatcher F. albicollis and T. ontarioensis from the collared flycatcher, T. paddae was not seen.

Key words: Trypanosoma - measurements - indices - owls - passerines

Danilewsky (1885) gave the first description of an avian trypanosome, Trypanosoma avium, from the owl Strix aluco. This trypomastigote had a striated appearance. In 1889, Danilewsky described T. avium majus and T. avium minus from an unspecified owl and the roller Coracias garrulus. He stressed the appearance of intermediates between the large and small forms and concluded that because of this, both belonged to the same taxon. Since that time, 96 nominal species and two varieties (Bishop & Bennett 1992) have been described from birds, the majority on the basis of the one host-one species philosophy; many of these species have a striated appearance. Initially, the morphology of the trypanosome was described in detail and absolute mensural values were employed in identification. However, it rapidly became apparent that these protozoa are highly pleomorphic and the use of indices was adopted to describe the morphology of the parasites (Hoare 1972). Recently, workers studying trypanosomes have used a variety of techniques as criteria for species identification rather than relying solely on morphological characters. Woo and Bartlett (1982) used characteristics of development of the parasites on specific media. Kirkpatrick et al. (1986) and Dirie et al. (1990) defined species on the basis of isoenzyme analysis and Kirkpatrick et al. (1986) also used lectin-binding and electrophoresis patterns while Molyneux and Gordon (1975) used cross immunity studies. These and other techniques are summarized by Apanius (1991). Others, such as Baker (1976), use host and/or vector specificity as a criterion. Such criteria may be satisfactory in some cases but Bennett (1961, 1970) demonstrated that T. avium [subsequently defined as T. confusum Lühe by Baker (1976)] could use two dipteran families as vectors and be transmitted to 11 species of birds representing seven avian families or subfamilies and four avian orders without change in morphology. Woo and Bartlett (1982) transmitted T. ontarioensis of crows to canaries with cultured material and Chatterjee and Ray (1971) transmitted T. avium bakeri from a bulbul to pigeons, chicks and quail. Bennett (1961) also showed that both large and small trypomastigotes could be produced in the same host as the result of a single inoculum and that these two morphs appeared in a somewhat cyclical fashion.

Avian trypanosomes appear, as a rule, in low intensities in their hosts and examinations of blood smears are unsatisfactory for detection of infections (Apanius 1991). Concentration techniques, such as the haemocrit centrifuge (Bennett 1962, Woo 1969) are far superior. However, modern techniques to identify the trypanosome species encoun-
tered are dependent on the availability and resources of a well-equipped laboratory, usually far-removed from the site of the collection of the host bird. Specimens in stained blood films are either identified only as Trypanosoma sp., regarded as a new species because it is in a different host, or given the name of a trypanosome previously described from that host species. None of these approaches to parasite identification in stained blood films are truly satisfactory.

The ability to experimentally transmit at least some species of avian trypanosomes to birds of widely differing phylogenetic relationships raises the question as to the validity of host specificity as a criterion for species diagnosis. It is possible that morphologically identical trypanosomes in widely differing avian hosts are the same species. If critical morphological studies show that trypomastigotes in different bird species and/or families are identical, then possibly the parasites represent only a single species.

In the period 1989-93, long term studies on the blood parasites of populations of pied and collared flycatchers (Ficedula hypoleuca, F. albicollis), great tits (Parus major) and Tengmalm's owl (Aegolius funerarius), among others, were instituted in various parts of Finland and Sweden. A number of these birds were infected with trypanosomes and these infections provided the material for a detailed study of the morphology of these parasites to determine whether morphologically similar forms appeared in different hosts. In the past, calculation of areas proved to be time consuming and were seldom undertaken. However, the advent of digital analyzing technology has made the calculation of areas much simpler. Therefore, the areas of the trypomastigote and derived indices have been included for the first time to determine the value of these criteria in the diagnosis of trypanosome species.

**MATERIALS AND METHODS**

Birds were sampled for haematozoa from five major locations in Fennoscandia. (i) The environs of the city of Uppsala, Sweden (pied flycatchers); (ii) the island of Gotland in the Baltic sea (collared flycatchers, great tits); (iii) Konnevesi in central Finland (pied flycatchers); (iv) Meltaus in Lapland province (pied flycatchers) and (v) in South Ostrobothnia in western Finland (Tengmalm's owl).

Blood smears were prepared from birds netted or trapped while incubating or feeding young. Blood smears were air-dried and fixed in 100% methanol. Some were stained locally in Sweden or Finland with one of the "quick" stains such as "Diff-Quik"; the remainder were stained at the International Reference Centre for Avian Haematozoa with Giemsa's stain at pH 7.2 and washed in acidic tap water of pH 6.5. Slides were examined with a Zeiss Ultraphot II microscope and those with more than 15 trypanosomes were saved for further study. Photomicrographs were taken with a Zeiss Photomicroscope III.

Trypanosomes were drawn with the aid of a camera lucida and the various measurements taken with a Zeiss MOP-3 Digital Analyzer. The measurements taken are as follows: PA = total length without free flagellum; PK = posterior end to kinetoplast; PN = posterior end to centre of nucleus; NA = centre of nucleus to anterior end; KN = kinetoplast to centre of nucleus; FF = free flagellum; BW = width of body through centre of nucleus; AT = area of trypomastigote; AN = area of nucleus; AK = area of kinetoplast. The indices derived from these measurements are: PK/PA, PN/PA, PN/NA (nuclear index), PN/KN (kinetoplast index), BW/PA (body width index) and AN/AT (nuclear area index). These measurements and indices are used throughout the text and tables. The free flagellum was frequently not stained sufficiently well to permit accurate measurement of its length.

**RESULTS AND DISCUSSION**

The prevalence of avian trypanosomes in blood smears from Fennoscandian birds was reported by Korpimäki et al. (1993 - A. funearius), Allander and Bennett (1994 - P. major) and Bennett et al. (1994 - F. hypoleuca). Trypanosomes from F. albicollis are unpublished data collected by Lars Gustafsson.

The trypanosomes are divided into three groups on the basis of gross morphology. The first group has striated trypomastigotes and are members of the T. avium complex; the group contains both large and small trypanosomes. The striated trypomastigotes (Figs 1, 2) from Tengmalm's owl (A. funearius) are defined as T. avium on the basis of Baker's (1976) comments that this species be restricted to trypanosomes from European owls (Strigidae). Striations appear in many accounts of avian trypanosomes and have been referred to as myonemes by some authors. However, these striations are in fact longitudinally arranged mitochondria (Molyneux & Robertson 1974). The second group (Figs 7-9) is completely consistent with Molyneux's (1973) description of T. everetti and is herein referred to as that species. The third group is represented by a small, non-striated trypanosome (Figs 10, 11), reminiscent of T. ontarioensis Woo and Bartlett, 1982 but is somewhat larger and has a shorter free flagellum. It is considered to be a member of the T. ontarioensis complex.
Figs 1, 2: *Trypanosoma avium* from Tengmalm's owl *Aegolius funereus*. Fig. 1: large. Fig. 2: small. Figs 3-6: *Trypanosoma avium* from the pied flycatcher *Ficedula hypoleuca*, showing range of sizes. Figs 7-9: *Trypanosoma everettii*. Figs. 7-8 from the great tit *Parus major*. Fig. 9: from the collared flycatcher *Ficedula albicollis*. Figs 10, 11: *Trypanosoma ontarioensis* from the collared flycatcher *Ficedula albicollis*. 
Trypanosoma avium complex (Figs 1-6)

The measurements and derived indices for trypanostigotes from Tengmalm’s (Boreal) owl (Table I) are considered to be those of *T. avium* and represent the measurements used for comparison with other striated avian trypanosomes. When the data published by Bennett (1961) for *T. avium* (Table II) is compared, it is seen that although both large and small morphs differed in absolute size, the indices are virtually the same. As Bennett’s (1961) material was also from an owl, *A. acadica*, then it is believed that the two sets of measurements refer to the same species, viz., *T. avium*. Furthermore, the occurrence of both large and small morphs of this species as described by Danilewsky (1889) supports this conclusion. Hence we reject Baker’s (1976) proposal to limit *T. avium* to Old World strigids and *T. confusum* to New World owls and reduce the latter species to synonymy. Baker (1956) identified a large striated trypanosome from the Corvidae as *T. avium* and his measurements (Table II) show that this trypanosome is identical to *T. avium* from both Old World and New World owls of the same genus. Baker (1976) later suggested that this trypanosome in the Corvidae was *T. corvi* Stephens and Christopher, giving a description for what was previously a nomen nudum. This suggestion is possibly in error as it is based solely on a presumed host specificity.

The striated trypanosomes (Figs 3-6) from the pied flycatcher *F. hypoleuca* (Table I) are classified into three groups on the basis of overall size. However, the ranges for the trypanosomes of each group (Table III) show that there is considerable overlap and the measurements form a continuum from the smallest to the largest; a similar overlap is seen between the pied flycatcher material and *T. avium* from Tengmalm’s owl (Table III). We conclude, therefore, that the striated trypanosomes with these range of measurements and indices are *T. avium*.

Trypanosoma everetti complex (Figs 7-9)

The small trypanosomes in the blood smears from the great tit *P. major* (Figs 7,8) and collared flycatcher *F. albicollis* (Fig. 9) are morphologically similar to *T. everetti* illustrated by Molyneux in 1973 from the blood of an African estrildid. The length measurements and derived indices (Table I) are also similar to those presented by Molyneux (1973) - Table II. However, additional measurements to those originally provided by Molyneux (1973) are reported herein. Although the two sets of measurements (Molyneux 1973 and this paper) were obtained from trypanosomes in different hosts from different continents, the close similarity in appearance and measurements of the two leads to the conclusion that these trypanosomes are indeed

### TABLE I

<table>
<thead>
<tr>
<th>Character</th>
<th><em>T. avium</em></th>
<th><em>T. avium</em> complex</th>
<th><em>T. everetti</em></th>
<th><em>T. ontarioensis</em></th>
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**TABLE II**

Measurements and derived indices of *Trypanosoma avium*, *T. everetti*, *T. ontarioensis* and *T. paddae*

<table>
<thead>
<tr>
<th>Characters</th>
<th>Trypanosoma avium</th>
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<th>T. ontarioensis</th>
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<td>24.2</td>
<td>18.9</td>
<td>16.0</td>
<td>8.8</td>
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<td>7.0</td>
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<td>3.0</td>
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<tr>
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<td>0.12</td>
<td>0.09</td>
<td>0.3</td>
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<tr>
<td>PK/PA</td>
<td>0.29</td>
<td>0.25</td>
<td>0.17</td>
<td>0.02</td>
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</table>

**TABLE III**

Comparison of the ranges of lengths and area of *Trypanosoma avium* from Tengmalm’s owl and small, medium and large trypanosomes from pied flycatchers. Data in μm and presented as mean with range in parentheses

<table>
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<th>Characteristic</th>
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<th>Trypanosoma avium - pied flycatcher</th>
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<tr>
<td></td>
<td>small</td>
<td>medium</td>
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<tr>
<td>PA</td>
<td>53.2 (37.8 - 69.0)</td>
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<td>9.1 (7.3 - 10.6)</td>
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<tr>
<td>BW</td>
<td>5.1 (3.2 - 6.4)</td>
<td>4.2 (2.3 - 6.2)</td>
</tr>
<tr>
<td>AT</td>
<td>150.1 (85.5 - 211.9)</td>
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<tr>
<td>AN</td>
<td>13.1 (8.8 - 19.1)</td>
<td>12.3 (6.1 - 17.8)</td>
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</table>

*T. everetti*. This trypanosome is one of the morphologically most unique of the avian trypanosomes (Baker 1976) and is readily identified in stained blood films. This trypanosome was also seen, in low numbers, in blood smears from collared flycatchers from Gotland and in pied flycatchers from Konevesi in Finland. *T. everetti* was first described by Molyneux (1973) from a west African estrildid and has subsequently been reported to occur throughout most of sub-Saharan Africa (Bennett et al. 1992). It was also reported in the willow warbler by Peirce and Mead (1984) in the United Kingdom. This record, together with the present study, suggests that this trypanosome has both a wide host range in unrelated avian hosts and a north-south Old World distribution. This distribution may well be the result of the north-south migration of European birds to Africa. As there is also considerable holarctic movement, the species may well be recorded in North America.

*Trypanosoma ontarioensis* complex (Figs 10, 11)

Woo and Bartlett (1982) described a small trypanosome they recovered from a crow as *T.
ontarioensis and which was unique among the avian trypanosomes because of its small size. A similar small trypanosome (Figs 10, 11) was seen in a blood smear from a collared flycatcher F. albicollis and the measurements and derived indices (Table I) were similar to those presented by Woo and Bartlett (Table II) for T. ontarioensis. While the absolute measurements (length, width and length of free-flagellum) are slightly greater than those presented by Woo and Bartlett, the derived indices are similar and the trypanosome from the collared flycatcher is considered to be T. ontarioensis.

A number of small trypanosomes have been described in the literature, particularly from birds of the Neotropics. Unfortunately, the descriptions are inadequate so that comparisons of length and indices can not be made. However, it is quite possible that all such small trypanosomes can be referred to T. ontarioensis. It would be worthwhile to make a full series of measurements on these small trypanosomes (especially from the original smears if these can be located) and compare them with T. ontarioensis. It appears on the basis of this study, that T. ontarioensis is a holarctic parasite. Its similarity to the Neotropical forms suggests that the distribution of this trypanosome is much broader than originally conceived (Woo & Bartlett 1982). If such a broad distribution does occur for this species, it is also possible that other species of trypanosomes have an equally broad global distribution.

None of the three species resemble T. paddae in that the measurements and derived indices (Table II) for this species are quite different and distinctive. T. paddae was considered as a possible trypanosome infecting birds of Fennoscandia as it has been recovered in boreal birds by both Bennett (1961) and Woo and Bartlett (1982).

Most of the 96 species of avian trypanosomes have been described on the assumption that they are specific at the avian host species level, regardless of their morphology or their similarity to already described species. Baker (1976) separated T. avium in Old World owls from T. confusum of New World owls based on the basis that they developed in, and were transmitted by, hippoboscid flies and would not develop in a variety of other blood-sucking arthropods, as did T. confusum nor would Baker's material infect other avian hosts. On the other hand, Chatterjee and Ray (1971) transmitted what they termed T. a. bakeri from the bulbul (Otocompsa = Pycnonotus) jocosus to pigeons, chicks and quail by inoculation of infected blood; all three host species became infected. This experiment supported those of Bennett (1961) by showing the lack of host specificity of these striated trypanosomes. Therefore, whether Baker's (1976) criterion for the recognition of trypanosome species is valid is debatable. Hoare (1972 loc. cit. pp. 116-117) in his monograph on the trypanosomes of mammals clearly indicates that many species of mammalian-inhabiting trypanosomes constitute morphologically indistinguishable biological races and strains differing only in antigenic structure (sero-demes), host range (xeno-demes) and impact upon the host (noso-demes). Thus T. cruzi in morphologically similar forms can occur in man, cats, dogs, pigs and a wide variety of other mammalian hosts; T. brucei has a number of sub-species or strains that occur in a variety of mammalian hosts (Hoare 1972). The work of Molyneux (1973) with T. bouffardi and Molyneux and Gordon (1975) on T. bouffardi, T. everetti and T. corvi suggests a similar situation also occurs in the avian trypanosomes. This would then explain why one morphological entity could be found in a wide range of avian hosts but it does not mean that each host has a separate trypanosome species. We conclude there are three morphologically distinct trypomastigotes in this sample of blood smears from Fennoscandian birds and these are represented by three species - T. avium, T. everetti and T. ontarioensis. Trypanosomes from other birds which have a similar morphology, measurements and derived indices can all probably be assigned to the same three species. Of course, confirmation by cross-transmission experiments and molecular or DNA fingerprinting studies are essential to confirm the validity of this hypothesis. However, until such technology is readily available to all scientists around the world, there will continue to be a reliance on mensural and morphological characteristics to provide the basis for species diagnosis among the avian trypanosomes. It is with this problem in mind that this paper has been presented to assist in the clarification of the identification of the species of trypanosomes.

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