A six-month duration, follow-up study of *Haemogregarina bigemina* at Foz do Douro, North Portugal, between ten and twenty years on

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Abstract

Haemogregarina bigemina infections were examined in the intertidal fish, *Lipophrys pholis*, at Foz do Douro, near Porto, North Portugal, over a period of six months between February and July, 2003. Prevalence of infection was 98.6% and intensity varied from 1-8% of erythrocytes parasitized. When tested statistically, intensity of infection was not related to host length, and intensity of infection for each parasitic stage varied significantly over the 6 months. Trophozoites were most common in March, suggesting that this might be a period of transmission. However, in the absence of annual intensity data and details of likely vector distribution, this observation is unconfirmed. Comparison of results with data reported between 10 and 20 years previously at the same site indicates that the characteristics of the haemogregarine infection have remained almost unchanged in this period.

Introduction

Haemogregarina bigemina Laveran and Mesnil, 1901 (Apicomplexa: Adeleorina) is a fish haemogregarine with an apparent broad host range and extensive geographical distribution (see Davies et al., 2004). The haematophagous, juvenile stages of at least two isopods of the genus *Gnathia* are probably its definitive hosts and likely act as its vectors (Davies, 1982; Davies & Smit, 2001). Investigations of *H. bigemina* in Portugal have included studies of its prevalence in fishes, its seasonality and transmission. In this region, *H. bigemina* has been demonstrated to infect shannies *Lipophrys pholis* (L.) (Osteichthyes, Blenniidae) at Foz do Douro (see Eiras, 1984; Sarasquete & Eiras, 1985; Eiras & Davies 1991) and at other sites along the Portuguese west coast (Eiras 1987; Davies et al., 1994). The parasite has also been reported from Montagu's blenny *Coryphoblennius galerita* (L.) (Osteichthyes, Blenniidae) (Davies et al., 1994; Davies et al., 2004) in Portugal.

Recently, the extraordinary longevity of *H. bigemina* infections among a host population (some 30 years) at a site in Wales was noted (see Davies et al., 2004.). For interest, this research note compares recent observations on *H. bigemina* from *L. pholis* captured at Foz do Douro, near Oporto, North

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Portugal with data collected from this host and site, 10 (Davies et al., 1994) and 20 years previously (see Eiras, 1984), and on several occasions in between these dates.

Materials and methods

Over a six-month period, between February and July 2003, 145 specimens of *L. pholis* (total length TL, 5.0 cm –17.8 cm) were captured with hand nets from rock pools at Foz do Douro, over a rocky surface of about 1,500 square meters. Fish were transferred to the laboratory in buckets of fresh seawater, where they were identified, anaesthetised with benzocaine (see Davies et al., 1994.), measured, and blood was collected from the caudal vein. Thin blood smears were fixed in absolute methanol and stained with May Grünwald-Giemsa. Infected smears were evaluated by calculating intensity of the infection for each parasite stage (trophozoite, meront, merozoite, immature and mature gamont), using random fields, an eyepiece grid and 100X oil-immersion objective. Possible relationships between intensity of the infection generally, intensity of infection with each parasitic stage, and the length of the host fish were evaluated by correlation analysis. Variation in intensity of the infection over the six-month period and intensity of infection by each parasitic stage were evaluated by a single factor ANOVA.

Results

Of 145 blennies examined, 143 (98.6%) were parasitized with *H. bigemina*. The two uninfected specimens measured 5.0 cm and 5.2 cm tail lenght (TL). In 93/143 (65.5%) of specimens the intensity of the infection was low, that is < 1% of erythrocytes infected. In 35/143 (24.5%) of fish, 1%-2% of the erythrocytes were infected. However, 12/143 (8%) of specimens had an intensity of infection between 2% and 3%, and 3 had intensity infection values of about 4%, 5% and 8%, respectively. Neither the intensity of the infection, nor the intensity of infection by the various parasitic stages was related statistically to the total length of the hosts. The results of an ANOVA analysis ($P = 0.36$; $a = 0.05$) also showed that there was no a significant variation in the intensity of the infection over the six months.

The parasitic forms observed within the erythrocytes (no intraleucocytic development was detected) corresponded to the known developmental stages of the parasite observed in the same host: trophozoites, meronts, immature gamonts and paired mature gamonts (see Davies et al., 2004.). Additionally, meronts undergoing binary division were observed, most dividing transversely, but some also by longitudinal fission. An ANOVA analysis showed a significant monthly variation in the intensity of infection of each parasitic stage [for $a = 0.05$: trophozoites ($P = 0.011$), meronts ($P = 0.005$), immature gamonts ($P = 8.0 \times 10^{-6}$) and mature gamonts ($P = 9.8 \times 10^{-5}$)]. Trophozoites and the immature gamonts were the least common forms in all six months, representing together less than 15% of the parasite population of infected erythrocytes. On the other hand, trophozoites were abundant in March, reaching about 15% of parasite population of infected red cells, and the immature gamonts were most common in February. The mature gamonts were most abundant in February and

March representing 26% and 44% of the parasite population, respectively. From April to June this value was between 16% and 19%, and in July it increased to 23%. Meronts were the most abundant stage for the whole six-month period, reaching a maximum value in June, where they represented 77% of the intraerythrocytic parasite population. Forms undergoing binary division were observed in all months, and were most common in April. In some host erythrocytes, nuclei were displaced by parasites, but in others, nuclei were modified in shape, becoming somewhat irregular. However, such changes were also observed in uninfected cells.

Discussion

Comparing the present data with that obtained from the same sampling site (Eiras, 1984; Sarasquete & Eiras, 1985; Eiras, 1987; Eiras & Davies, 1991; Davies et al., 1994) it appears that the general characteristics of the infection have not changed. The developmental stages of the parasite in *L. pholis* are the same as those already reported at Foz do Douro, as well as for other locations such as France (Laveran and Mesnil, 1901), Wales (Davies & Johnston, 1976; Davies, 1982) and South Africa (Smit & Davies, 1999; Davies & Smit, 2001).

The prevalence of the infection (98.6%) is identical to that reported by Eiras & Davies (1991) (98%), but higher than that recorded by Sarasquete & Eiras (1985) (83%), Eiras (1987) (78.6%), and Davies et al. (1994) (23.0%). However, the sample populations of Sarasquete & Eiras (1985), Eiras (1987) and Davies et al. (1994) included many small fish below 5.0 cm long. For instance, Davies et al. (1994) sampled 66/91 (72.5%) *L. pholis* less than 5.0 cm TL. *Haemogregarina bigemina* is usually apparent in *L. pholis* measuring between 3.2 cm (Eiras, 1987) and 3.5 cm long (Davies & Johnston, 1976). By the time these fish approach 5.0 cm (Davies & Johnston, 1976) to 7.0 cm long (Eiras, 1987), all are infected. Consequently, if large numbers of smaller blennies are sampled, overall prevalence will appear reduced. However, if only *L. pholis* of 5cm and above are considered (probably 1 year and older, see Davies & Johnston, 1976) as in the current study (5.0 – 17.8 cm TL) and in Eiras & Davies (1976) (5.1– 15.5 cm TL), it can be concluded that the prevalence of infection at Foz do Douro has been maintained at very high and almost identical levels (98 – 99%), for some years.

In contrast, when the intensity of the infection (parasitaemia) is compared with past studies at Foz do Douro, some differences in observations emerge. Except in one instance (a fish showing 8% infected red cells), the present study shows intensity values smaller than those reported by Sarasquete & Eiras (1985) (up to 6% erythrocytes infected), but higher than those referred to by Eiras (1987) (usually <0.01% of erythrocytes infected), Eiras & Davies (1991) (between <0.1%-2.5% infected red cells) and Davies et al. (1994) (between <0.1%-0.3% erythrocytes parasitized). These differences are difficult to account for, but may be related to variations in vector abundance, or in cell counting methods. In the current study, a grilled eyepiece, not used previously, perhaps allowed more precise determinations of intensity. On the other hand, no direct relationship between host length (and

therefore, host age) and infection intensity was detected, supporting the observations of Eiras (1987) and Eiras & Davies (1991) in Portugal, and Davies & Johnston (1976) and Davies (1982) in Wales.

The distribution of the parasitic stages over six months showed some differences with the seasonality study of Eiras & Davies (1991). In the present study meronts were the most prevalent stage, while for those authors these were the stages encountered least often. However, Eiras & Davies (1991) did not test the seasonality of the infection statistically, while our current results showed significant variation in the different parasitic stages over six months, despite all parasitic stages being observed every month. The fact that the trophozoites were most abundant in March suggests that this may be the month that transmission of the infection may occur. However, in the absence of annual data and information about likely vectors on this occasion, it is impossible to draw firm conclusions. In Wales, females of *L. pholis* lay eggs between April and August, metamorphosed juveniles (3-4cm long) occur from July to October and the first patent infections with *H. bigemina* are found from September in fish of 3.5 cm long and upwards, indicating that transmission must occur in summer and early autumn (see Davies & Johnston, 1976). Seasonality seems to be important for the development of some fish haemogregarines such as *Desseria* (*Haemogregarina*) *platessae* (Lebailly, 1904) Siddall, 1995 (see Laird & Morgan, 1973), *Desseria* (*Haemogregarina*) *nototheniae* (Barber et al., 1987) Siddall, 1995 (see Barber et al., 1987) or *Haemogregarina georgianae* Barber & Westerman, 1988 (see Barber & Westerman, 1988), but seasonality has not been reported for *H. bigemina* (see Davies, 1995). Clearly more observations are needed to clarify this point.

The present observations are interesting because they were undertaken on a fish population at a Portuguese sampling site used between 10 and 20 years previously (Eiras, 1984; 1987; Sarasquete & Eiras, 1985; Eiras & Davies, 1991; Davies et al., 1994). Such long-term observations of haemogregarine populations are rare, and they indicate that the ecological conditions that promote transmission of infection, in this case the likely abundance of the presumptive vectors, gnathiid larvae (see Smit & Davies, 2004), and those that favour the interaction between fish and the isopod, have remained relatively unchanged over a significant period.

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