Haemosporidian infections in captive exotic glossy starling *Lamprotornis chalybaeus* in Hong Kong

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Abstract. A greater blue-eared glossy starling *Lamprotornis chalybaeus* Ehrenburg from a large flight aviary in Hong Kong was found on post mortem to be infected with *Plasmodium octamerium* Manwell, 1968, *Plasmodium* cf. *relictum* (Grassi et Feletti, 1891) and *Haemoproteus* cf. *pastoris* Mello, 1935. Descriptions of their morphology are provided as none of the examined parasites fully concord with their type (or neotype) material descriptions. *Plasmodium octamerium* has been recorded in avian hosts from geographically distant locations, suggesting that infection in imported hosts may persist in a chronic state for a long period. This *Plasmodium* species as well as *P. relictum* are evidently not fastidious in choice of passeriform hosts and are transmitted by ubiquitous domestic mosquito vectors, apparently facilitating their proliferation among zoo and aviary inhabitants. The *Haemoproteus* infection appears to be conspecific with *H. cf. pastoris* reported from a myna (*Acridotheres tristis*) in Singapore. Mynas are also common in Hong Kong, which suggests a possible cross-transmission of infection between these two starlings.

A greater blue-eared glossy starling *Lamprotornis chalybaeus* Ehrenburg of African origin, obtained from an aviary in Germany and kept for 10 years in an aviary in Hong Kong, died on 16 October 2005. Three haemosporidian parasites were identified from blood smears prepared ante and post mortem: *Plasmodium octamerium* Manwell, 1968, *Plasmodium* cf. *relictum* (Grassi et Feletti, 1891) and *Haemoproteus* cf. *pastoris* Mello, 1935.

Manwell and Rossi (1975) in their communication on blood protozoa of imported birds to the USA list *P. octamerium* as well as *P. circumflexum* and *P. nucleophilum* from *L. chalybaeus*. Bennett and Herman (1976) report infections with *Haemoproteus sturni* (syn. of *H. pastoris* – see Valkiunas 1997), *P. relictum* and a species of *Leucocytozoon* in *L. chalybaeus* from Kenya. Ashford et al. (1976) report infections with *Haemoproteus* sp., *Plasmodium relictum*, and *Leucocytozoon* sp. in Ethiopia.

In the present communication, morphology of the reported parasites is described and possible routes of transmission among exotic captive birds are discussed.

MATERIALS AND METHODS

The starling's blood was examined prior to (withdrawn from the brachial vein) and after its death (withdrawn from the heart). Blood films were fixed in absolute methanol and stained with Giemsa (15% in phosphate buffer, pH 7.2) for 1 hour. Microscopic examination included a check of at least 50,000 erythrocytes at $\times 1,000$ (oil immersion) magnification. For *H. cf. pastoris* was calculated the Nuclear Displacement

Ratio (Bennett and Campbell 1972) – $2X / X+Y$ (X = distance between the erythrocyte wall and its nucleus; Y = distance between the erythrocyte wall and its nucleus on the infected side).

RESULTS

Description of the parasites

Plasmodium (Giovannolaia) octamerium Manwell, 1968 Figs. 1–14

Parasites were seen usually in mature erythrocytes, exceptionally (trophozoites) in reticulocytes. They neither deformed the erythrocyte, nor displaced its nucleus. The level of parasitaemia was 4‰ (calculated from 6,080 erythrocytes); 46% were trophozoites. Trophozoites appeared alongside the erythrocyte nucleus, but did not touch it. They already contained several pigment granules (Figs. 1–4). In young meronts, half of their volume was occupied by the single nucleus, and their cytoplasm contained many translucent vacuoles (Fig. 5). The only meront with two nuclei traced, was longer than the erythrocyte nucleus and was extending alongside it. This meront contained an aggregate of pigment and three vacuoles staining conspicuously blue (Fig. 6). Only few meronts with eight or less nuclei were traced (Figs. 7, 8). Differentiated meronts (segmenters) contained 10–16 nuclei (i.e., a progeny of 10–16 merozoites), arranged into a string of paired nuclei, extending alongside and exceeding in length the host nucleus (Figs. 9, 10); few rounded up into a polar position (Fig. 11). The chain of nuclei was usually interrupted by a

Figs. 1–11. *Plasmodium octamerium* from *Lamprotornis chalybaeus*. **Figs. 1–4.** Trophozoites. **Fig. 5.** Young uninucleate meront. **Fig. 6.** Meront with two nuclei. **Figs. 7, 8.** Meronts with up to eight nuclei. **Figs. 9, 10.** Mature schizonts with 14–16 nuclei. **Fig. 11.** Meront with 16 nuclei in polar position. P – pigment; V – vacuole.

cytoplasmic segment where pigment aggregated, often accompanied by one or more vacuoles (globules), staining conspicuously faint blue (Figs. 7–9).

The gametocytes were elongate, exceeding the erythrocyte nucleus in length. The mid-part of both the macro- (Figs. 12, 13) and microgametocytes (Fig. 14) was inflated and either displaced the erythrocyte nucleus or descended to a polar position. The pigment was either aggregated or scattered.

Plasmodium (Haemamoeba) cf. relictum (Grassi et Feletti, 1891) Figs. 15–23

Infection was sparse; parasitaemia was 0.5‰ (trophozoites, if present, could not be distinguished from *P. octamerium*). Observed meronts contained 5 to 8 nuclei leaving in between wide patches of cytoplasm (Figs. 15–19). Pigment either aggregated in patches or in scattered grains. The meronts and particularly the gameto-

Figs. 12–14. *Plasmodium octamerium* from *Lamprolornis chalybaeus*. **Figs. 12, 13.** Macrogametocytes. **Fig. 14.** Microgametocyte in polar position. n – nucleus.

cytes occupied up to about three quarters of the erythrocyte space, while displacing the nucleus to apical or lateral position; in the process the erythrocyte rounded up (Figs. 20–23).

One erythrocyte contained a nine-nucleate *P. cf. relictum* meront and a microgametocyte of either *P. cf. relictum* (more likely) or *P. octamerium*; the erythrocyte nucleus was strongly displaced to lateral position.

***Haemoproteus cf. pastoris* Mello, 1935 Figs. 24–31**

Infection was low and detected only in the set of blood films drawn ante mortem. The blood films contained only macrogametocytes in several stages of differentiation. The erythrocyte became enlarged from an early stage of infection (Figs. 25, 26). Young ellipsoid macrogametocytes were $6.0\text{--}6.4 \times 2.4 \mu\text{m}$ (Figs. 25, 26), those slender, elongate were $8.7\text{--}9.6 \times 1.8 \times 2.2 \mu\text{m}$ (Figs. 27–29) and those large, mature were $10.7\text{--}11.8 \times 2.1\text{--}3.3 \mu\text{m}$ in size (Figs. 30, 31). With maturation, the infected erythrocyte became increasingly deformed and its nucleus became displaced. The nuclear displacement increased from a ratio of 1 in young ellipsoid-stage infection to 0.53–0.37 in infections of slender stages to 0.33–0.30 in erythrocytes with differentiated (or mature) macrogametocytes.

DISCUSSION

***Plasmodium octamerium*.** It was first described in a bird of African origin, *Vidua macroura* (Pallas), of the family Estrilidae, obtained from a pet shop in the USA (Manwell 1968). Manwell's (1968) experimental infection of canaries yielded about 80% segmenters with 8 nuclei. In a different experimental host, *Spizella arborea*, infection consisted of many segmenters with 10, over 12 to over 16 merozoites. Valkiunas (1997) in his account on *P. octamerium* from Manwell's neohapantotype (Nos. 645 and 646) from "*Sturnus* sp." (= *L. chaly-*

baeus?) reports meronts with 6–24 merozoites. Neither Manwell (1968) nor Valkiunas (1997) mention the presence of faint-blue-staining vacuoles. Such vacuoles could, however, become less distinct in long stored specimens. Among species of the subgenus *Novyella* the blue-green-staining body was described as a globule due to its refractory properties (Mohammed 1958); in our *P. octamerium* the vacuoles or globules were not conspicuously refractile. Valkiunas (1997) also reports infections in immature erythrocytes, not mentioned earlier. The flagellum-like appendices, although not mentioned in earlier descriptions are not exceptional among species of the subgenera *Giovannolaia* and *Novyella* (Valkiunas 1997). In spite of several discrepancies between the available descriptions and the presently described parasite, it is most likely *P. octamerium*.

***Plasmodium cf. relictum*.** A recent molecular study confirmed that *P. relictum* is not fastidious in choice of passeriform hosts (Martinsen et al. 2006). Molecular data, however, also yielded ambiguous results: at least two distinct lineages were attributed to *P. relictum* isolates: GRW4 (Valkiunas et al. 2007) and SGS1 (Gill H. and Paperna I., unpubl.; Valkiunas G.A., pers. comm.). More molecular studies will be required to identify the boundaries between species included in the subgenus *Haemamoeba* Garnham, 1966 and the extent of conspecificity between infection with agents of morphology similar to that of *Plasmodium relictum* (see Landau et al. 2003) from diverse hosts.

***Haemoproteus cf. pastoris*.** The gametocytes from the glossy starling blood were identical with *H. cf. pastoris* reported from a myna (*Acridotheres tristis*) in Singapore (Paperna et al. 2005, 2007). The gametocytes from both hosts differ from *H. pastoris* from *Sturnus roseus*, the type host (and other starlings, see Valkiunas 1997), mainly in having a heavy load of coarse pigment granules and in extremely displacing the erythrocyte nucleus already from premature stage of development.

Figs. 15–23. *Plasmodium* cf. *relictum* from *Lamprolornis chalybaeus*. **Figs. 15–19.** Meronts. **Figs. 20, 21.** Macrogametocytes. **Figs. 22, 23.** Microgametocytes.

Infection in captive birds: caged pets, aviaries and Zoos. *Plasmodium octamerium* has been reported both from native *L. chalybaeus* (Bennet and Herman 1976) and from imported pet birds – *Vidua macroura*, its type host (Manwell 1968), and *L. chalybaeus* (Manwell and Rossi 1975, present communication). Its appearance in geographically widely separated avian hosts suggests that infection in the imported hosts may persist in a chronic state for a long period (perhaps years). Nonethe-

less, the possibility that the infection was imported with its host from Germany 10 years ago is very remote. There are no data suggesting what species of culicine mosquitoes are capable of transmitting *P. octamerium*. Many species of avian *Plasmodium* are, however, non-fastidious in their choice of vectors and are capable to be transmitted by ubiquitous domestic mosquitoes (*Culex pipiens*) and in Hong Kong likely by *Culex quinquefasciatus* (Hong Kong Government 2005).

Figs. 24–31. *Haemoproteus* cf. *pastoris* from *Lamprotornis chalybaeus*. **Fig. 24.** Non-infected erythrocyte. **Figs. 25, 26.** Young macrogametocytes. **Figs. 27–29.** On-growing slender and elongate macrogametocytes. **Figs. 30, 31.** Mature macrogametocytes grossly distorting the host erythrocyte.

In Manwell and Rossi's (1975) list, *P. relictum* was considered rare, reported in only one bird. Fatal incidences due to *P. relictum* (or *P. relictum*-like species) hyperparasitaemia have been observed among captive birds (Beier et al. 1981). Infections with *P. relictum* were linked to mortalities among Zoo penguins (Stoskopf and Beier 1979, Graczyk et al. 1994). Available data, including molecular studies (McConkey et al. 1996), leave us uncertain as to whether the penguin parasites are conspecific with *P. relictum* found among passerine birds (Valkiunas 1997, Landau et al. 2003). The apparent low species specificity of *P. relictum* to avian hosts together with its capability of being trans-

mitted by ubiquitous domestic vectors (such as *C. pipiens*) (see Valkiunas 1997) increases the risk of its proliferation among Zoo and aviary inhabitants.

The apparent conspecificity between the *Haemoproteus* infection agent from Singapore myna and that from the Hong Kong glossy starling *L. chalybaeus* suggests a cross-transmission between these two starlings. Myna is common in Hong Kong; it is an invasive bird which expands its distribution range throughout the warm zones of Asia, Africa and the Pacific.

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