Pathology and first report of natural infections of the eye trematode *Philophthalmus lachrymosus* Braun, 1902 (Digenea, Philophthalmidae) in a non-human mammalian host

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The avian eye trematode Philophthalmus lachrymosus *Braun, 1902 is for the first time referred naturally occurring in a non-human mammalian host. Previously, natural infections with* P. lachrymosus *and other species of* Philophthalmus *have been occasionally reported from man, with few data on experimental infections of non-human mammals. Results presented here are related to the report of two cases of philophthalmosis due to natural infections of wild Brazilian capybaras,* Hydrochaeris hydrochaeris *L., 1766 with* P. lachrymosus *and associated pathology. Clinical signs, gross and microscopic lesions as well as new morphometric data on the parasite are presented.*

Key words: Digenea - *Philophtalmus lachrymosus* - mammals - *Hydrochaeris hydrochaeris* - pathology

The capybara (*Hydrochaeris hydrochaeris* Linnaeus, 1766), the largest rodent in the world, distributed in Panama, Colombia, the Guyanas and Peru, south through Brazil, Paraguay, NE Argentina, and Uruguay (Woods 1993) has been often investigated for endo or ecto parasites. This animal either in wild environments or in captivity is regarded as an important reservoir for the outspreading of wild zoonoses induced by acari, bacteria, helminths, protozoans, and viruses (Munoz & Chaves 2001, Figueiredo et al. 1999, Ito et al. 1998, Sinkoc 1997, Arias et al. 1997, Lemos et al. 1996, Casas et al. 1995, Franke et al. 1994, Lombardero et al. 1983).

This paper deals with the first cases of natural infections of a non-human mammalian host, the capybara, with avian eye trematodes of the genus *Philophthalmus* Looss, 1899 with the associated pathology.

MATERIALS AND METHODS

Two cases of eye parasitism with helminths were detected in one adult male and one newborn female wild capybaras (*H. hydrochaeris*), weigh 30,400 g and 17,200 g, respectively, autochthonous in Foz do Iguaçu (25º32'45"S, 54º53'07"W), state of Paraná, Brazil.

Parasites were removed from the palpebral conjunctiva with the aid of a thin brush (no. 00), briefly rinsed in a 0.85% NaCl solution and fixed compressed/uncompressed in cold A.F.A (alcohol 70º GL, 93 ml; formaldehyde, 5 ml; acetic acid, 2 ml); some were stained with carmine, dehydrated in an ethanol series, cleared in phenol and preserved as whole mounts in beechwood creosote and balsam (1:3, respectively). Other samples were kept as wet material in the fixative solution. A small piece of an infected palpebral conjunctiva was surgically removed from one animal under intramuscularly administered anesthetic (an association of tiletamine hydrochloride 4 mg. kg^{-1}, zolazepam hydrochloride 4 mg. kg^{-1}, xilazine hydrochloride 0.4 mg. kg^{-1}, atropine sulphate 0.04 mg. kg^{-1}) and immediately fixed in 10% formalin. The excised portion was then routinely processed for paraffin embedding (Behmer et al. 1976). Five micrometers thick sections were stained with hematoxylin and eosin (HE).

Whole mounts, wet material and histological sections were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC).

Ecological terms are in accordance with Bush et al. (1997). Taxonomic status of the digenetic parasite species follows Freitas (1955) and Ching (1961); classification of mammals is in accordance with Woods (1993) and Groves (1993), that of the birds is after Pinto (1978) and Sick (2001). Measurements are in millimeters (mm) and means are in parentheses. Photomicrographs were obtained in a Zeiss Axiopht bright-field microscope with a DIC (Differential Interference Contrast) apparatus. The development of this research has been authorized by the IBAMA no. 1/41/94/0499-4 for the Itaipu Binacional Wild Animal Center.

RESULTS

The brief description is based on 10 compressed specimens.

Philophthalmus lachrymosus Braun, 1902
(Figs 1-7)

Body 3.4-4.25 (3.73) long, 0.85-1.53 (1.08) wide. Oral sucker sub-terminal, 0.22-0.29 (0.26) long, 0.26-0.33

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(0.30) wide. Ventral sucker (acetabulum) bigger than the oral sucker, median, pre-equatorial, 0.61-0.72 (0.67) long, 0.63-0.77 (0.70) wide. The ratio between the suckers is 1:2, respectively. Pharynx 0.19-0.21 (0.20) long, 0.09-0.15 (0.11) wide. Genital pore bifurcal, 0.63-0.84 (0.71) from the anterior end. Cirrus pouch elongate, 1.26-1.96 (1.67) long, extending from the genital pore to the post-acetabular zone. The cirrus pouch contains an elongate and sometimes sinuous seminal vesicle, short prostatic region and also, sinuous and elongate muscular cirrus. Testes slightly lobate, post-ovarian, inter-cecal, in the posterior portion of the body. Anterior testis 0.14- 0.27 (0.23) long, 0.28-0.46 (0.37) wide; posterior testis 0.15-0.36 (0.28) long, 0.28-0.47 (0.36) wide. Ovary generally rounded, smooth, median, pre-testicular and post-uterine, 0.15-0.28 (0.19) long, 0.15-0.29 (0.21) wide. Uterus mostly pre-glandular. Operculate eggs with eye-spotted miracidium, thin-shelled, 0.090-0.11 (0.10) long, 0.021-0.040 (0.030) wide. Vitellaria with a few follicles, distributed along a thin collecting duct that extends to 71-90% (78.6%) of the distance between the anterior testis and the acetabulum. Excretory pore terminal.

Taxonomic summary
Host: *Hydrochaeris hydrochaeris* L., 1776, Rodentia, Hydrochoeridae, common names: capybara, "capivara".
Other hosts: *Larus dominicanus* Lichtenstein, 1823, Charadriiformes, Laridae; common names: Kelp gull, "gaivotão"; *Larus maculipennis* Lich. 1832; common names: gull, "gaivota"; *Casmerodius alba* (Gmelin, 1789) [= *Casmerodius albus egretta* (Gmelin, 1789)], Ciconiiformes, Ardeidae; common names: great egret, "garça-branca"; *Nyctanassa violacea* (Linnaeus, 1758), Ciconiiformes, Ardeidae; common names: yellow-crowned night heron, "sabacu, savacu, tamatião, matirão"; *Thalasseus maximus* (Boddaert, 1783) (=*Sterna maxima* Boddaert), Charadriiformes, Laridae, common names: royal tern, "trinta-réis grande"; *Catoptrophorus semipalmatus* (Gmelin, 1789), Charadriiformes, Charadriidae, common name: willet, semipalmated snipe; *Homo sapiens* L., 1758 (accidental host).
Site of infection: palpebral conjunctiva.
Locality: Foz do Iguaçu, state of Paraná, Brazil.
Deposited specimens: CHIOC no. 36563 a-h (whole mounts), 35399, 35400, 35401 (wet material). The slide 36563h contains histological cross-sections.

Clinical signs were represented by ocular secretion, blindness and emaciation. Gross lesions consisted of opacity of the cornea, anemic mucosa with adhered parasites; other lesions of the palpebral conjunctiva were represented by diffuse and multiple millimetric (about 3 mm) nodular bright-whitish formations together with a discrete congestive process (Fig. 1). Parasites were generally located in the conjunctival inner and outer ocular canthus and in the inferior conjunctiva. Low worm burdens were mostly observed in the ocular canthus. The microscopic lesions consisted of papillar projections with scamous epithelial cells (Fig. 4). In the center of the papillae feeble vessels were observed. Frequently, trematodes were found attached to these projections by the ventral sucker; this attachment provoked a remarkable constriction of the papillae (Figs 5, 6, 7). Caliciform cells, although absent in the areas of cellular proliferation were well observed around these sites. The stroma presented a discrete diffuse mononuclear inflammatory infiltrate and hyperemia.

Fig. 1: multiple nodules in the palpebral conjunctiva of a capybara infected with specimens of *Philophthalmus lachrymosus*. Bar = 10 mm.

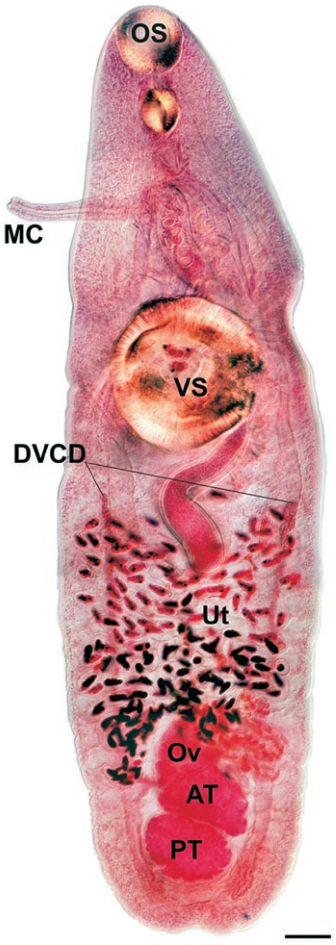

Fig. 2: *Philophthalmus lachrymosus*. Total. OS: oral sucker. MC: muscular cirrus. VS: ventral sucker. DVCD: distal vitellaria collecting duct. Ut: uterus. Ov: ovary. AT: anterior testis. PT: posterior testis. Bar = 0.2 mm.

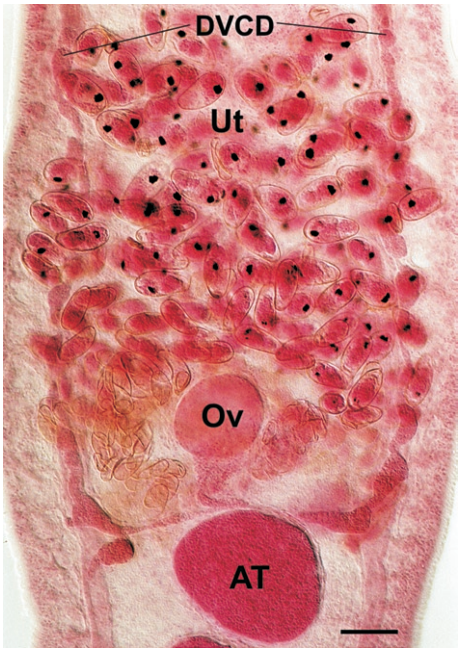

Fig. 3: *Philophthalmus lachrymosus*. Detail of the vitellaria region, showing the distal ends of the collecting duct (DVCD) from the border of the anterior testis (AT), encircling the ovary (Ov) and the uterus (Ut). Bar = 0.1 mm.

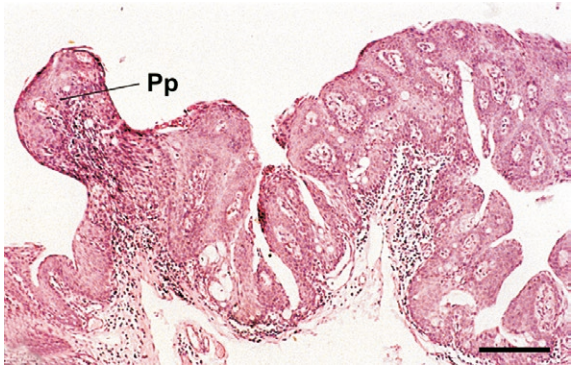

Fig. 4: cross-section of the palpebral conjunctiva of a capybara infected with *Philophthalmus lachrymosus*. Papillar projections (Pp) centralized by blood vessels. HE. Bar = 0.2 mm.

Fig. 5: cross-section of the palpebral conjunctiva of a capybara infected with *Philophthalmus lachrymosus*. Longitudinal section of a parasite with the ventral sucker attached to a papilla (Pp). HE. Bar = 0.5 mm.

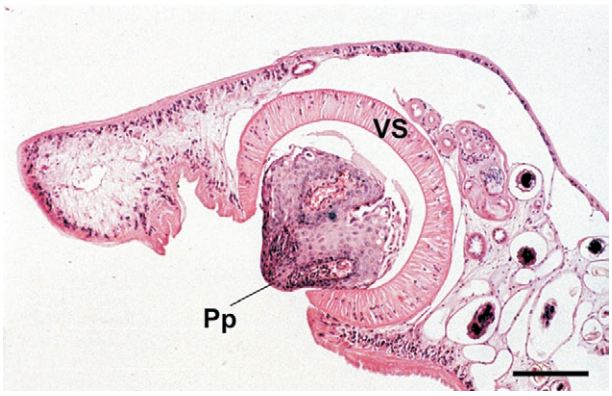

Fig. 6: cross-section of the palpebral conjunctiva of a capybara infected with *Philophthalmus lachrymosus*. Detail of the ventral sucker (VS) and the encircled papilla (Pp) HE. Bar = 0.2 mm.

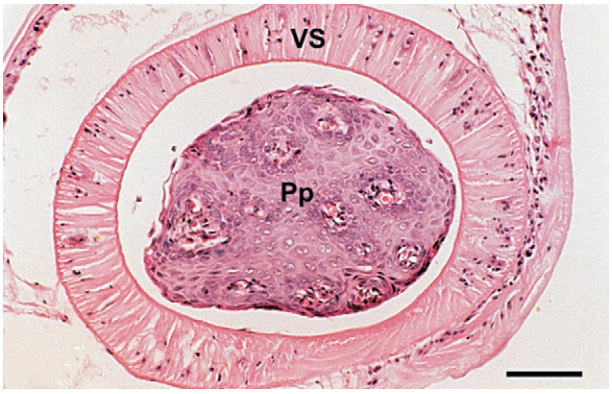

Fig. 7: cross-section of the palpebral conjunctiva of a capybara infected with *Philophthalmus lachrymosus*. Transversal section of the ventral sucker (VS) encircling a papilla. HE. Bar = 0.08 mm.

DISCUSSION

Philophthalmus lachrymosus, proposed by Braun (1902) on the basis of Brazilian samples recovered from the eyes of the gull, *Larus maculipennis* Lichtenstein, 1823, was redescribed by Freitas (1955) in a study of eye parasites of the great egret *Casmerodius albus egretta* (Gmelin, 1789), also reporting to *Nyctanassa violacea* (L.) and *Thalasseus maximus* (Boddaert) as hosts for the parasite. The species was further referred in the conjunctival sacs of the Kelp gull *Larus dominicanus* Lich., 1823 by Travassos et al. (1960), in the optical cavity of *Catoptrophorus semipalmatus* (Gmelin, 1789) by Nasir and Diaz (1972) and in the eyes of humans (Nollen & Kanev 1995, Lamothe-Argumedo et al. 2003).

Brief morphometric data on specimens of *P. lachrymosus* recovered from capybaras are provided here since differences, mainly related to the overall smaller dimensions of the worms, were observed in the comparison of those previously described parasitizing birds. These changes are probably due to the adaptation of the parasite to the mammalian host.

Considering the fact that there are 37 valid species in the genus, Nollen and Kanev (1995) affirm that several

species of *Philophthalmus* Looss, 1899 fall into special groups and will need more study to determine their validity. Also, Freitas (1955) during his study of *P. lachrymosus* observed differences in the specimens, when compared to data after Braun (1902) and considered that the differences were related to age, compression of specimens and low host specificity that could interfere in the aspect and size of morphological structures.

In accordance with Nazir and Diaz (1972), "…insofar as the identity of various species is concerned, the only character, which seems to substantiate is the point of size, and where life histories are known, is the larval morphology". Moreover, the type of vitellaria is not constant for all species.

According to Ávila-Pires (1989), the parasite shift from one host to another implicates in several mechanisms and complex adaptations that depend on behavior patterns, synchronization of activities and interspecific relations, in a long-term co-evolutionary process. Thus, the hosts, acting as biological filters are able, during this process, to induce the expression of the phenotypic plasticity of the parasites, as strategies for their survival against the immunological responses of the parasitized organisms.

The capybaras from Foz do Iguaçu are in close contact with wild birds that are represented by Ciconiiformes, Charadriiformes, Lariformes, Anseriformes and Podicipediformes birds that act as definitive hosts for *P. lachrymosus* and thus as potential vectors for the spreading of the philophthalmosis among the capybaras in that locality. Previous data on the development of philo-phthalmid worms in non-human mammals were based on experimental infections only (Alicata & Ching 1960, Karim et al. 1982).

In relation to intermediate hosts, the life cycle of *P. lachrymosus* remains unknown. Zhongzhang et al. (1980), dealing with another species of the genus, *P. gralli* Mathis and Leger, 1910, that is very close to *P. lachrymosus*, observed cercariae of that parasite naturally occurring in the prosobranch thiarid snail *Melanoides tuberculatus* (Müller, 1774), encysted on plants or on the shells of various species of planorbids. In the study area, several Prosobranchia and Pulmonata snails are present and can also act as potential intermediate hosts for *P. lachrymosus*.

Taking into account the suitable conditions of transmission on what concerns the definitive and intermediate hosts besides environmental aspects of the study area, accidental human philophthalmosis can be settled by means of contaminated waters with cercariae or encysted metacercariae in the surroundings (Alicata & Ching 1960).

The clinical signs observed in the infected capybaras consisted of severe conjunctivitis with blindness; these signs were more intense than those reported in human philophtalmosis, devoid of either outstanding clinical signs or lesions. In such cases, mild keratoconjunctivitis, redness, ocular irritation, watery eyes, edema of the semilunar fold, and proliferation of papillae in the palpebral conjunctiva were observed (Mimori et al. 1982, Gutierrez et al. 1987, Lang et al. 1993, Lamothe-Argumedo et al. 2003). The latter alteration was remarkable in the capybaras and seems to be characteristic of the infection in mammals. Nevertheless, Kanev et al. (1993) surveying several cases of human infections with pre-adults of *P. lucipetus* Rudolphi, 1819, refer to blindness affecting a woman in Germany, with eight specimens of the parasite in the orbital cavities. Thus, the probable low pathogenicity observed in human infections may be related to the small size of worm burdens, that in most of the cases consist of a single worm. In birds heavily infected, edema of the nictitating membrane, persistent conjunctival inflammation with intense eye watering, irritation and ocular congestion have been reported (Richter et al. 1953, West 1961, Alicata 1962, Greve & Harrison 1980, Bhatia et al. 1985). However, Howell (1971) observed that experimentally infected chickens with up to 20 parasites of *P. burrili* Howell & Bearup, 1967 did not present clinical signs. Thus, also in birds, high worm burdens seem to determine the intensity of the pathological alterations. The microscopic lesions present in the palpebral conjunctiva of the capybaras associated to the presence of *P. lachrymosus* were severe, in despite of the discrete inflammatory reaction and could have served as an entry to secondary infections due to bacteria, fungi or viruses thus inducing the blindness in the animals. The few data on the microscopic lesions due to species of *Philophthalmus* are those referred in birds with similar but milder alterations when compared to the observed in the present study (Howell 1971, Schmidt & Toft 1981).

Ferreira (1973) suggests that the parasitic diseases could be interpreted as accidents in the search for a steady equilibrium between parasite and host due to modifications in one of the elements in the parasite-host-environment system. On the basis of the present findings these modifications seem to be occurring, taking into account that mature specimens of *P. lachrymosus* were found parasitizing an unusual host, with heavy worm burdens, responsible for the cases of philophthalmosis reported here.

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