J. Comp. Path. 2013, Vol. 148, 294–297

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INFECTIOUS DISEASE

Disseminated Sparganosis in a Cynomolgus Macaque (*Macaca fascicularis*)

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Summary

An adult male cynomolgus macaque (*Macaca fascicularis*) from Mauritius arrived at our facility in France after a 1-year period of quarantine in Spain. Clinical examination soon after arrival revealed the presence of numerous firm cutaneous and subcutaneous nodules (0.1–0.5 cm diameter) in the scrotal and inguinal areas, and persistent mild eosinophilia. On necropsy examination additional similar nodules were found in the peritoneum and abdominal wall, omentum and mesentery. Microscopical examination revealed disseminated eosinophilic granulomas containing tapeworm larvae identified as *Spirometra erinaceieuropaei* by direct sequencing of the *cox1* gene.

Keywords: eosinophilia; *Macaca fascicularis*; sparganosis; *Spirometra*

The presence of opportunistic parasites is a common incidental finding on necropsy examination of laboratory macaques. Such parasites are most often discovered in the stomach and large intestine (Drevon-Gaillot *et al*., 2006; Chamanza *et al*., 2010) and their presence is often anecdotal and of no consequence. However, they may cause immunological disturbances, making it impossible to use the affected animal in most experimental studies. We describe here a significant infestation of a cynomolgus macaque (*Macaca fascicularis*) with a parasite of the genus *Spirometra*.

A batch of seven cynomolgus macaques were purchased by our facility in June 2010. The animals originated from Mauritius and had spent 1 year in a quarantine centre in Catalonia, Spain. Shipment to and maintenance at our facility conformed to French national regulations. The Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA) facilities have full authorization for use of non-human primates (agreement number 92 032 01) and are inspected regularly by national veterinary inspectors. During quarantine, each animal was treated twice with praziquantel (5 mg/kg, last treatment in September 2009) and five times with ivermectin (0.5 mg/kg, every month from June to October 2009 and just before shipment in March 2010). After shipment to our facility, the animals were assigned to infectious disease studies and housed in biosafety level 3 rooms. In September 2010, a mass was discovered at the site of an axillary lymph node biopsy in one adult male (>7 years) in good bodily condition (body weight 7 kg). Subsequent full clinical examination revealed a right axillary subcutaneous mass (1 cm diameter) and numerous firm cutaneous and subcutaneous nodules (0.1–0.5 cm diameter) in the scrotal and inguinal areas, together with diffuse, moderate erythema and a swelling of the scrotum.

Haematological examination revealed a slightly elevated leucocyte count (12.6×10^9/l; reference range $5–10 \times 10^9$/l) with moderate neutrophilia (8.6×10^9/l; reference range $2–7 \times 10^9$/l) and mild eosinophilia (0.6×10^9/l; reference range $0.05–0.4 \times 10^9$/l). Fine-needle aspiration of the axillary mass was performed for cytological examination

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2012.08.003

and biopsy samples were collected from the inguinal and scrotal masses for histological examination. Biopsy samples were fixed in 10% neutral buffered formalin and processed routinely.

The animal was treated with praziquantel (5 mg/kg, one dose) and amoxicillin (15 mg/kg for 5 days). In December 2010, 2 months after treatment, the animal was examined again. At this time the right axillary mass had disappeared, but the scrotal and inguinal masses were still present. Haematological analysis revealed a normal leucocyte count (6.5×10^9/l) with moderate eosinophilia (0.9×10^9/l). These abnormal results prevented inclusion of this macaque in immunological studies and, as drug-resistant sparganosis was suspected, the animal was humanely destroyed. A complete necropsy examination was performed. Tissue samples were either fixed in 10% neutral buffered formalin for histological examination or were snap frozen in liquid nitrogen for polymerase chain reaction (PCR) analysis. Fixed samples were processed routinely, embedded in paraffin wax and sections were stained with haematoxylin and eosin (HE) and Von Kossa's stain for calcium.

Microscopical examination of the inguinal and scrotal biopsy samples showed well delineated and encapsulated chronic subcutaneous and cutaneous inflammatory lesions 50 µm to 8 mm in diameter (Fig. 1). At the centre of each focus was a metazoan organism surrounded by a ring of macrophages, some of which were large and angular with indistinct cell borders (epithelioid macrophages) or contained several randomly located nuclei (foreign body giant cells), together with neutrophils and eosinophils (Fig. 2). At the periphery of the lesions there was a fibrotic capsule with admixed lymphocytes and plasma cells. The overlying epidermis, when present, displayed mild and focal uniform hyperplasia. The degree of inflammation varied between lesions. The

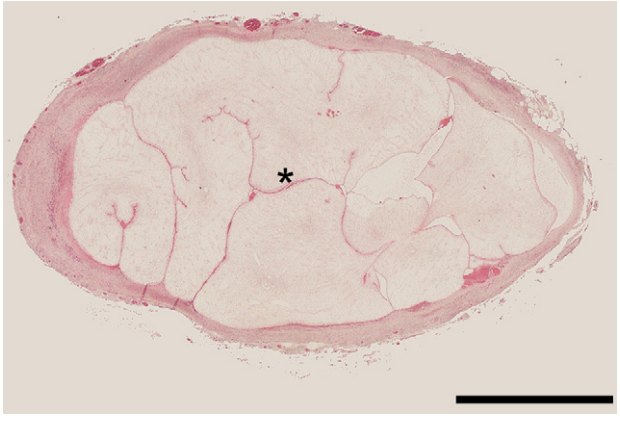

Fig. 1. Skin. Well delineated and encapsulated granuloma enclosing a tapeworm larva (asterisk). HE. Bar, 1 mm.

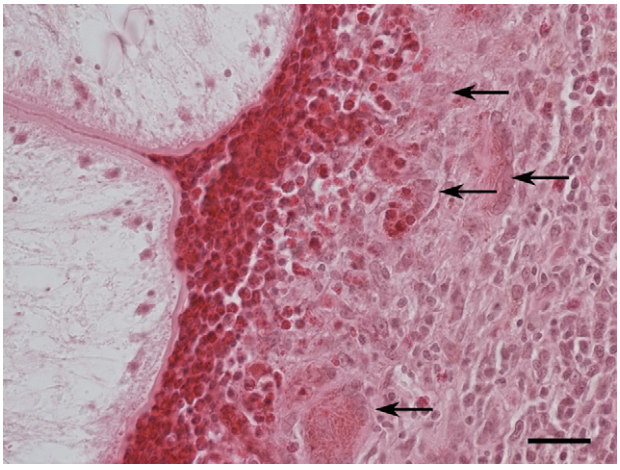

Fig. 2. Ring of multinucleated giant cells (foreign body type; black arrows) and epithelioid macrophages admixed with neutrophils and eosinophils. HE. Bar, 100 µm.

parasite (Fig. 3) was a curled acoelomic worm with an amorphous eosinophilic cuticle, loose internal mesenchyme and muscle fibres suggestive of a tapeworm larva. Von Kossa's stain (Fig. 3, inset) revealed the presence of numerous oval calcareous bodies in the larval mesenchyme. On the basis of these observations, a diagnosis of multifocal, chronic and moderate, eosinophilic and granulomatous dermatitis/panniculitis with intralesional tapeworm larva was made.

Cytological smears of the fine-needle aspirate from the right axillary mass revealed a subacute neutrophilic lymphadenitis correlated with the observed blood neutrophilia.

On necropsy examination, numerous nodules were present in the inguinal and scrotal skin. Additionally,

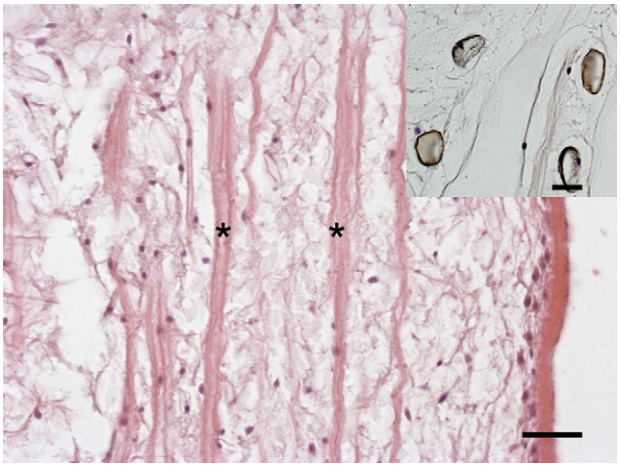

Fig. 3. Tapeworm larva with an eosinophilic cuticle and internal loose mesenchyme and muscle fibres (asterisks). HE. Bar, 40 µm. Inset: Calcareous bodies. Von Kossa's stain. Bar, 15 µm.