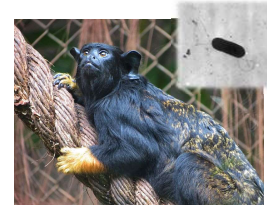


FATAL SEPTICAEMIC LISTERIOSIS IN A GOLDEN-HANDED TAMARIN (*SAGUINUS MIDAS*)



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Introduction

Ubiquitous *Listeria monocytogenes* can cause a rare and severe food-borne disease in vertebrates, including humans, who develop different clinical signs and lesions. In ruminants, rhombencephalitis and meningoencephalitis are the main lesions. However, humans can also present with abortion, gastrointestinal disease and septicaemia. We report a case of sudden death in a captive golden-handed tamarin (*Saguinus midas*) due to septicaemic listeriosis.

Material and Methods

A captive 10-year-old male golden-handed tamarin was found dead without prior signs of illness. Necropsy was carried out with toxoplasmosis, yersiniosis, and bacterial/viral hepatitis as differential diagnoses. Tissue samples were collected for histopathology, bacteriology and parasitology. For histopathological examination of liver, lung, spleen, kidney and encephalon, samples were fixed in 10% buffered formalin and embedded in paraffin using standard procedure. Five-µm thick sections were stained with hematoxylin and eosin (H&E) and afterwards by Gram staining and examined using light microscopy (Olympus BX60 and Image Focus Alpha[®] camera).

Bacteriology: samples consisting of intracardiac blood (hemoculture), a piece of lungs, liver and spleen were aseptically collected and inoculated in culture media for bacteriological examination. Hemoculture was dispensed into Brain Heart Infusion Broth (BD, USA) and the organs (except the intestine), after maceration in physiological saline, were inoculated onto Columbia Agar Base with 5% sheep blood and MacConkey Agar (Biomérieux, France) and incubated at 36±1°C for 18/24 hours.

Parasitology: fresh brain tissue was homogenized for DNA extraction on a fully automated KingFisher™ Flex Purification System (Thermo Scientific) for *Toxoplasma gondii* DNA detection targeting the 35-fold repetitive glycerol-3-phosphate dehydrogenase (B1) gene (Hohlfeld *et al.* 1994) by conventional PCR; the Willis flotation method was used for the diagnosis of gastrointestinal parasites.

DNA extraction on formalin-fixed and paraffin-embedded liver and brain tissues was performed for *L. monocytogenes* PCR diagnosis and molecular serogroup determination by multiplex PCR according to described by Kerouanton *et al.*, 2010.

Results

Gross examination revealed pulmonary congestion and discrete white spots in the liver.

Histology showed necrotic foci with mononucleated cells and numerous Gram-positive coccobacilli in liver (Figure 1) and spleen (Figure 2). Clusters of these bacteria were also detected in glomerular and interstitial renal vessels as well as in alveolar capillaries (Figure 3). Non-suppurative meningitis (severe infiltration by lymphoplasmacytic inflammatory cells with scarce polymorphonuclear cells) and granulomatous encephalitis (perivascular cuffing and gliosis foci, some of them forming small granulomas, mainly in brainstem, cerebellum, thalamus and cerebral cortex) with intralesional Gram-positive coccobacilli was also observed (Figure 4).

PCR of hepatic and brain tissue detected *L. monocytogenes* (4b serogroup) (Figure 5).

Conventional PCR for *Toxoplasma gondii* detection resulted negative. A small intestinal infection by nematodes (Order *Ascaridida*) was identified. The sample material for bacteriological analysis was heavily contaminated with *Proteus*.

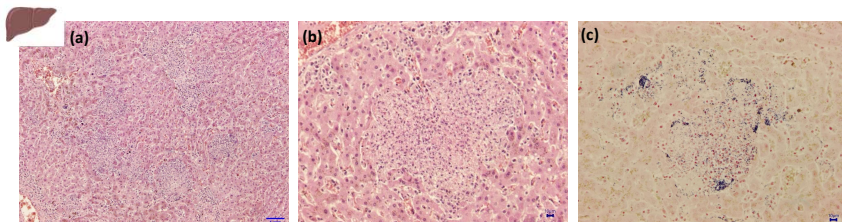


Figure 1. Liver : (a) and (b) Necrotic foci with mononucleated cells (H&E) ; (c) Numerous Gram- positive coccobacilli in the necrotic foci.

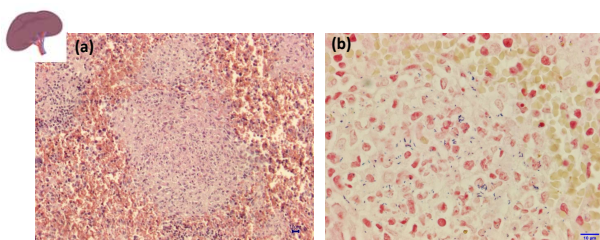


Figure 2. Spleen: (a) Necrotic foci (H&E) ; (b) Gram-positive coccobacilli in the necrotic foci.

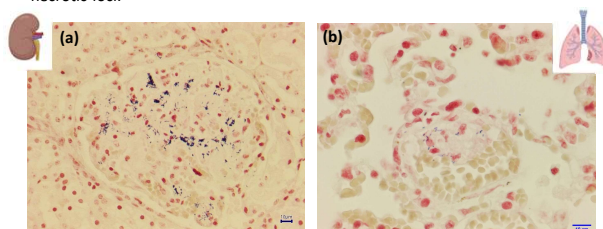


Figure 3. Gram -positive coccobacilli observed in the renal glomerule (a) and alveolar capillaries (b).

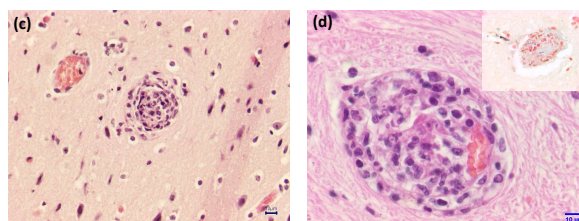
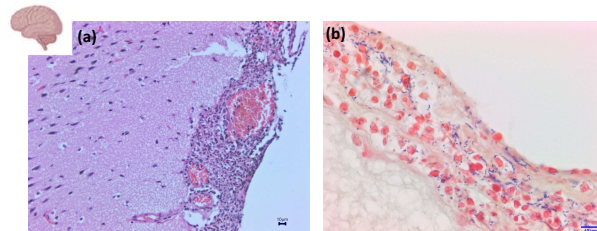


Figure 4. Brain (at level of thalamus): (a) Non-suppurative meningitis (H&E) with intralesional Gram-positive coccobacilli (b); (c) Granulomatous foci in grey matter (H&E) and perivascular cuffing (d) (H&E) with few Gram-positive coccobacilli (inset).

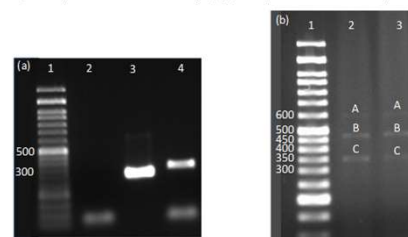


Figure 5. (a) Electrophoresis gel of the PCR for the identification of *Listeria monocytogenes*. Lane 1 – Ladder VI; Lane 2 – Blank; Lane 3 – Positive control *Listeria monocytogenes* CECT 934; Lane 4 – Golden-handed tamarin sample. (b) – Electrophoresis gel of the PCR for the identification of *Listeria monocytogenes* serogroups. Lane 1 – Ladder VI; Lane 2 & 3 – Sample with the three bands visible corresponding to the serogroup 4b (A– 597 bp; B – 471 bp; C – 370 bp).

Conclusions

We report the first case of septicaemic listeriosis in a golden-handed tamarin caused by *L. monocytogenes* 4b serogroup, a serogroup frequently detected in human clinical isolates. The results show that listeriosis should be included in the differential diagnosis of bacterial hepatitis and meningoencephalitis in this species and possibly primates in general.