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Systemic listeriosis in caged Canaries (*Serinus canarius*)

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Cavp-2007-0185.R1**Systemic listeriosis in caged Canaries (*Serinus canarius*)****Olatunde B. Akanbi[¶], Angele Breithaupt¹, Ulf Polster¹, Thomas Alter², Anette Quandt³, Andreas Bracke³ and Jens P. Teifke^{1*}**

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Abstract

The occurrence of listeriosis in 12 caged canaries is described where 50 % of the birds including the female and all the offspring died within two weeks without clinical signs. At necropsy, multifocal necrotising and partly granulomatous hepatitis, splenitis, myocarditis, interstitial nephritis, and exudative pericarditis with intra-lesional *Listeria monocytogenes* were the predominant findings as shown by histopathology and immunohistochemistry. Microbiology, serology and PCR revealed *L. monocytogenes* serotype 1/2a as the causative agent. Thus listeriosis has to be considered in the differential diagnosis for granulomas associated with mycobacteriosis, yersiniosis, coligranulomatosis or fungal infections.

Introduction

Although listeriosis is a well known bacterial infection of poultry (Seastone, 1935; Paterson, 1937), the occurrence of this disease under modern management conditions seems to be rather rare. *Listeria monocytogenes* organisms are widely distributed Gram-positive, facultative anaerobic intracellular bacteria that might be a commensal organism of clinically healthy animals and are ubiquitous in the environment, stream water, dust, sewage and soil (Huff *et al.*, 2005). There are more than 13 serotypes but most of the infections in animals are related with serotypes 1/2a, 1/2b, and 4b (Peters & Born-Stapp, 1997; Nadon *et al.*, 2001). *L. monocytogenes* is transmitted by ingestion of food, and water, e.g. contaminated with faeces or vaginal discharges from aborting animals. Listeriosis has been reported to cause disease in a broad spectrum of animals including mammals, especially ruminants, birds, fish and even poikilotherms, and thus is regarded a public health concern being a food borne disease for humans (Southwick & Purich, 1996)

The most common avian hosts are chickens, geese and canaries (Hatkin *et al.*, 1986) but listeriosis has also been recorded in grouse, partridge, and snowy owl and an adult cockatiel (Gray, 1958, Shivaprasad *et al.*, 2007). In poultry, listeriosis usually has a septicaemic course and affects predominantly heart, liver and spleen. In these organs, *L. monocytogenes* induces gross lesions of necrotising hepatitis myocarditis, splenitis and myocardial degeneration with pericarditis (Gray & Killinger, 1966). Listerial encephalitis is a rather uncommonly reported lesion in birds, including chickens and a merlin (Baker, 1967; Kurazono *et al.*, 2003; Cooper, 1992). Sporadic outbreaks of listeriosis have been

described in poultry and birds such as parrots, budgerigars and canaries but in most case reports no details were given concerning clinical signs and gross pathology.

This report describes clinical, gross, histopathological, immunohistological and microbiological findings seen during an enzootic listeriosis in an aviary and discusses listeriosis as differential aetiological diagnosis for granulomatous hepatitis and splenitis in canaries.

Materials and methods

Case history. In July 2007, two juvenile canaries were submitted for necropsy. They had been housed in an aviary measuring 4m×2m×2m with 10 other canaries (male and female canaries with 10 offspring from two broods in 2007), four cockatiels and two guinea pigs. According to the owner's history the aviary roof was covered by a wire netting, not excluding the possibility that feral birds could defecate into the cage. The birds in the aviary were fed with grain and fruit and were given water for drinking and bathing. The cockatiels were occasionally fed with pieces of cheese. Notably, in this year the nest used for the first canary brood (five offspring) was immediately reused by the female for the second brood (five offspring) without cleaning in between. Inspection of the aviary revealed a corner littered with decomposed food material admixed with sprouted grain. All birds were healthy until the first mortalities in the second brood occurred during the last two weeks of June 2007. The owner observed sudden depression in affected birds, separation from the group, prostration and flightlessness but with no loss of appetite. The

day following the first signs the birds succumbed. Finally, all five canaries from the second brood and the adult female canary subsequently died. Since the owner was concerned about highly pathogenic avian influenza (HPAI) as a potential cause for this “mass mortality”, the last two birds were submitted for necropsy to the Friedrich-Loeffler-Institut.

Bacteriology. Samples of lung, liver, spleen, kidney, heart and intestine were plated on Columbia agar (OXOID, Wesel, Germany) with 5 % bovine blood and Water-blue Metachrome-yellow Lactose Agar (Gassner Agar, SIFIN, Berlin, Germany) at 37 °C and cultured aerobically and anaerobically at 37 °C for 24 to 48 h. The intestinal contents were cultured in Salmosyst broth base with Salmosyst selective supplement (Merck, Darmstadt, Germany) at 37°C for 24 h, and thereafter subcultured on Gassner Agar at 37°C for at least 48 h. Presumptive colonies of *Listeria* spp. were differentiated using the API-Listeria system (bioMerieux, Nürtingen, Germany) and the Christie, Atkins, Muench-Petersen (CAMP) reaction. Examination for *Chlamydia* was carried out with smears from liver and spleen and a commercially available immunofluorescence reagent (bioMerieux).

Histopathology. Samples of liver, spleen, myocardium, kidney, lungs, musculature, skin, and brain were fixed in 10% neutral buffered formalin, processed and embedded in paraffin-wax; sections (4 µm) were cut and stained with haematoxylin and eosin (HE).

Immunohistochemistry. Rabbit polyclonal antibody against *Listeria* O antigen (Difco, Detroit, MI; 2302-50-0) was used as described earlier (Ramos-Vara & Beissenherz, 2000). The antibody was diluted 1:200 in Tris-buffered-saline and the avidin-biotin-peroxidase complex (ABC) method with amino-ethyl-carbazole substrate chromogen (DakoCytomation, Hamburg, Germany) was used including positive controls and negative control serum. The sections were slightly counterstained with Mayer's haematoxylin.

Serotyping and PCR analysis. *L. monocytogenes* strains were serotyped by the method of Seeliger and Hohne (1979) using commercial *Listeria* antisera (Mast Assure *Listeria*, Mast Diagnostics, Merseyside, UK) according to the manufacturer's instructions but with minor modifications. Briefly, for the somatic (O) antigen, isolates were grown on tryptose agar (Merck) for 24 h at 37 °C. A positive reaction consisted of agglutination of the isolate after at least 1 min of mixing with one drop (approximately 10 µl) of serum. The polyvalent sera OI/II and OV/VI were used first and if there was a positive reaction with OI/II, OI and OI/IV sera were then used. A positive reaction with OV/VI was followed by testing with sera OVI, OVII, OVIII and OIX. For the flagellar (H) antigen, isolates were grown on tryptose agar for 24 h at 37 °C, inoculated into 5 ml tryptose broth (Merck), and incubated for 24 h at 20°C. One ml of broth was used to inoculate tryptose motility agar (Merck) which was incubated for 24 h at 20°C. One drop of sera (A, AB, C and D) were mixed with 5 µl of motility-agar material. A positive result was indicated by agglutination.

In addition isolates were tested using a multiplex PCR which identifies serotypes 1/2a, 1/2b, 1/2c, and 4b. For this *L. monocytogenes* chromosomal DNA was extracted using a DNA extraction kit (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany). The primers and PCR conditions were as previously described (Doumith *et al.*, 2004). As controls, a set of five strains was used: serotype 1/2a DSM 12464, serotype 1/2b NCTC 7973, serotype 3c SLCC 2479, serotype 4b ATCC 19115 and serotype 6a ATCC 33090.

Results

Gross findings. Postmortem examination revealed the carcasses to be moderately fleshed, with vents and feathers matted with greenish-white watery faeces (Fig. 1A). The livers of both canaries exhibited multiple slightly elevated pale yellow nodules with caseous material on the cut surface. These lesions were up to 3 mm in diameter, sharply demarcated although partly merging and were interpreted as granulomatous and caseous necrotising hepatitis. Bird no. 2 had a serofibrinous exudate in the pericardium and air sac (Fig. 1B), with congestion of the apical parts of both lungs. The spleen of bird no. 1 had few yellow areas up to 1 mm diameter while in bird no. 2 the moderately enlarged spleen (6 mm x 3 mm x 3 mm) displayed numerous pinpoint white-yellow foci (interpreted as necrosis) (Fig. 1C). The kidneys were pale and slightly swollen with scattered grey areas (Fig. 1D). Both canaries had catarrhal enteritis with green faeces.

Microscopic findings. In both birds, approximately 40% of the hepatic parenchyma was replaced by numerous concentrically laminated granulomas (0.2 to 1.0 mm in diameter) that often elevated the overlying capsule (Fig. 2A). The inflammatory foci contained a central intensely eosinophilic area admixed with karyorrhectic cellular debris (caseous necrosis) and were rimmed by numerous epithelioid macrophages and multinucleated giant cells (Langhans type macrophages). These areas were surrounded by a narrow band of fibroblasts and collagen (fibrosis) (Fig. 2B). There was multifocal periportal infiltration with plasma cells, lymphocytes, macrophages, and rare multinucleated giant cells. In bird no. 1 there was diffuse severe acute fibrinous pericarditis, epicarditis, epicardial steatitis and multifocal myocardial coagulative necrosis with heterophilic and monocytic perivasculitis and vasculitis. The lung showed a severe acute diffuse congestion and oedema and an accumulation of a protein-rich fibrinous exudate in the distended alveoli. A diffuse mild interstitial infiltration with lymphocytes and histiocytes was present.

Within the spleen (no. 2) there was diffuse loss of lymphoid cells and follicles (moderate lymphoid depletion) with multifocal degeneration and necrosis of the parenchyma with accumulation of a homogeneous eosinophilic partly beaded material (oedema and fibrin). The kidneys showed focal necrosis of tubular epithelial cells with interstitial infiltration with numerous lymphocytes and plasma cells and fewer heterophils (necrotising and interstitial nephritis) (Fig. 2C). Apart from congestion, no lesions were seen in the cerebrum and cerebellum of either bird.

The ABC-method immunohistochemistry revealed strongly positive staining for *L. monocytogenes* antigen within kidney (Fig. 2D), liver (Fig. 2E), spleen, and

myocardium. Intralesionally, there were large numbers of *L. monocytogenes* in the cytoplasm of phagocytic cells, mainly in macrophages and Kupffer cells in the liver including few hepatocytes (Fig. 2E, inset). Using the rabbit control serum no staining was obtained within the granulomas.

Microbiological findings. The microbiological examination revealed large quantities of weakly β -haemolytic, catalase-positive, CAMP-positive, and Gram-positive rods in lung, liver, spleen, kidney, heart and intestine in both birds, mixed with a few *Citrobacter youngae* organisms in bird no. 1. The Gram-positive isolate was identified as *L. monocytogenes* with the profile number 6510 obtained using the commercial *Listeria* identification system API Listeria (bioMerieux). Serological testing of *Listeria monocytogenes* isolates yielded serovar 1/2a and this was confirmed by multiplex PCR (Doumith *et al.*, 2004).

Tests for *Chlamydia* spp. and *Salmonella* spp. gave negative results and acid fast bacteria were excluded by treatment of touch preparations of the hepatic granulomas with "TB fluorescence stain kit T" (Difco). Tracheal-swabs were tested for HPAIV H5N1 but proved negative. Based on these results the aetiology for this systemic granulomatous and necrotising inflammation in canaries was determined to be infection with *L. monocytogenes*.

Discussion

Systemic listeriosis has been observed in neonates of several species including calves, lambs, and foals. As in mammals, young birds appear to be more susceptible to *Listeria* infection than adults (Gray, 1958). In the present cases, the hepatic portal areas were more severely affected, suggesting that the bacteria reached the liver via portal blood circulation.

Epidemiological studies provide strong evidence that both sporadic and common-source outbreaks of Listeriosis are food-borne (Southwick & Purich, 1996). We therefore could not exclude the possibility that food, e.g. cheese feeding of the cockatiels, could have posed an entry risk for the agent. The presence of listerial septicaemia in some species including HIV infected human beings may indicate an underlying disease process that results in immunosuppression. However, we were not able to demonstrate concurrent infections or other diseases. We therefore hypothesise that the stress of recurring broods by the adult female canary and the poor hygiene in the aviary (i.e., contaminated soil, environment, nest boxes, bedding, and feed) could have increased the susceptibility of the birds to infection.

In conclusion, this case report suggests that the canary may be more susceptible to *L. monocytogenes* than the cockatiel. Earlier published data also suggests that gross and histological lesions may vary in different avian species despite the same serotype of *L. monocytogenes* being involved (Peters & Born-Stapp, 1997; Shivaprasad *et al.*, 2007). Shivaprasad *et al.* (2007) described widespread coagulative necrosis in the cockatiel, whereas the canaries described in this case report showed multifocal necrosis with formation of granulomas in association with exudation in the serosal cavities. Similar

findings were described in canaries by Peters and Born-Stapp (1997). However, the distribution of immunostaining and microbiological results revealed septicaemia consistently in all birds. Thus, systemic listeriosis can also lead to granulomatous inflammation that has to be differentiated from lesions after infections with *Mycobacterium* spp., *Yersinia pseudotuberculosis*, *Salmonella* spp., *E. coli*, or *Aspergillus* spp.

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Figure legends

Fig. 1 (A) Canary no. 1. Young bird in poor body condition. Translucent through the body wall, within the body cavity there is a yellow mass (arrow). Note soiled feathers from diarrhoea (asterisk) (B) Canary no. 2. The liver has multiple sharply demarcated nodules up to 3 mm in diameter. Note diffuse serofibrinous pericarditis. (C) Canary no. 1. Liver: Multifocal to coalescent granulomatous and necrotising hepatitis and perihepatitis. Spleen: Miliary necrotising splenitis. (D) Canary no. 2. Kidney: Enlarged and pale tan kidneys with numerous pinpoint-sized white foci.

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Fig. 2 (A) Canary no. 1. Liver: multiple areas of granulomatous hepatitis with concentrically lamellated caseous necrosis. Bar = 500 μ m. (B) Canary no. 1. Liver: granuloma composed of central caseous necrosis, admixed with multinucleate giant cells, rimmed by lymphocytes, epithelioid macrophages and a thin collagenous capsule. Bar = 200 μ m. (C) Canary no. 2. Kidney: focal necrosis of tubular epithelium with lymphoplasmacytic interstitial nephritis. Bar = 100 μ m. (D) Canary no. 2. Kidney: Immunohistochemical detection of intra-lesional *L. monocytogenes* antigen, consecutive section to C. ABC-method. Bar = 100 μ m. (E) Canary no. 1. Liver: Strong positive immunostaining for *L. monocytogenes* antigen within epithelioid macrophages beneath the fibrous capsule of a granuloma. ABC-method. Bar = 200 μ m. (Inset) The staining is confined to single rod shaped intracellular bacterium. ABC-method. Bar = 30 μ m.

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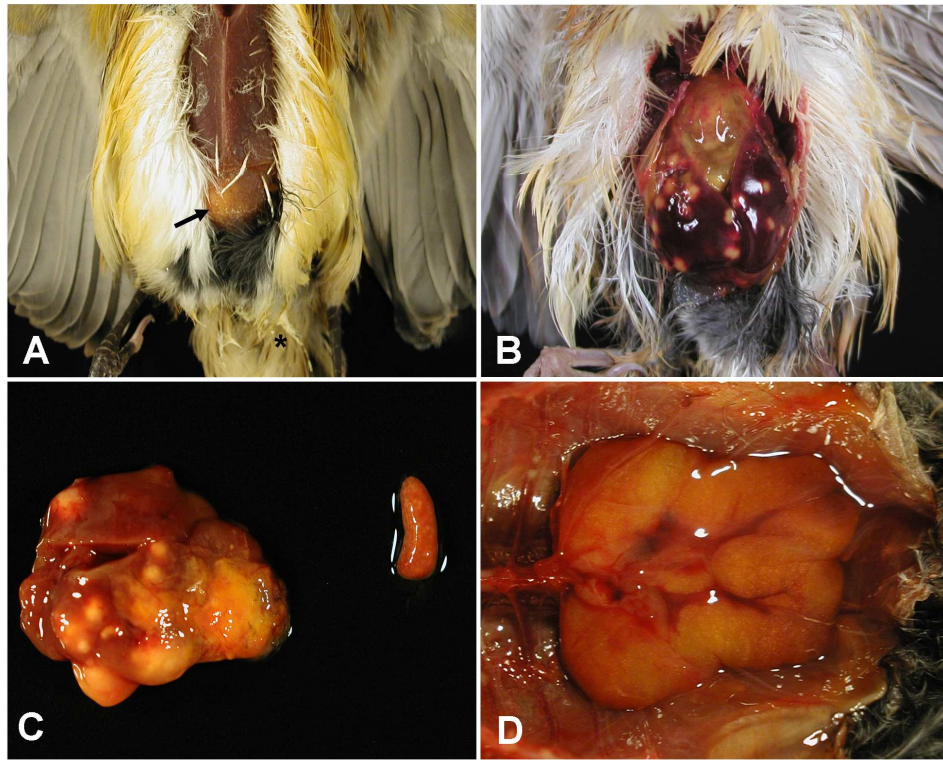


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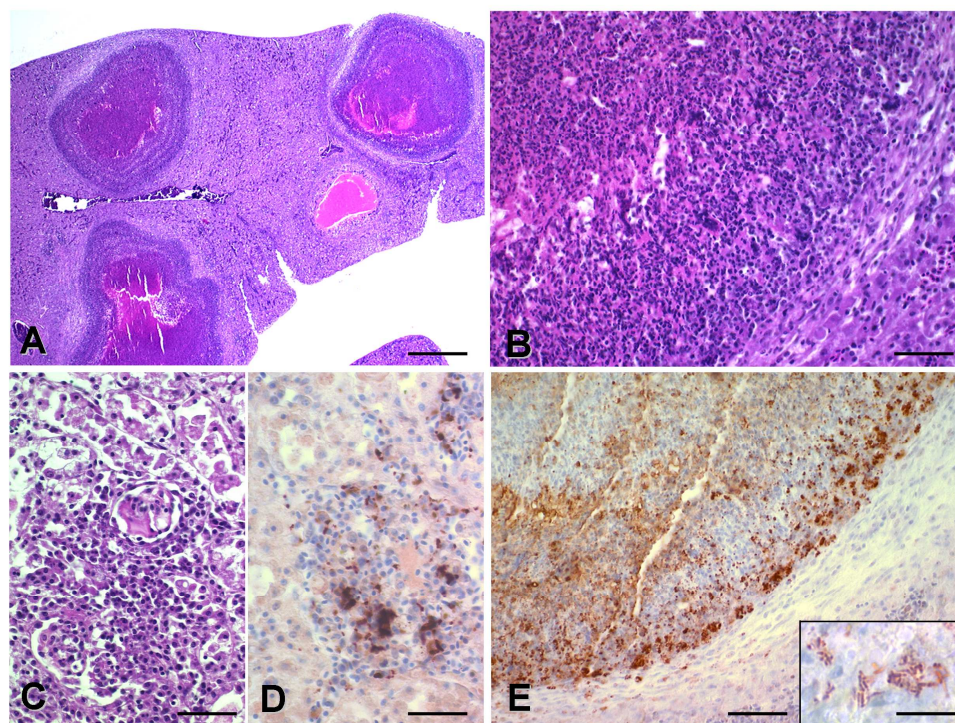


Fig. 2 (A) Canary bird no. 1. Liver: multiple areas of granulomatous hepatitis with concentrically lamellated caseous necrosis. Bar = 500 μ m. (B) Canary bird no. 1. Liver: granuloma composed of central caseous necrosis, admixed with multinucleate giant cells, rimmed by lymphocytes, epithelioid macrophages and a thin collagenous capsule. Bar = 200 μ m. (C) Canary bird no. 2. Kidney: focal necrosis of tubular epithelium with lymphoplasmacytic interstitial nephritis. Bar = 100 μ m. (D) Canary bird no. 2. Kidney: Immunohistochemical detection of intralosomal *L. monocytogenes* antigen, consecutive section to C. ABC-method. Bar = 100 μ m. (E) Canary bird no. 1. Liver: Strong positive immunostaining for *L. monocytogenes* antigen within epithelioid macrophages beneath the fibrous capsule of a granuloma. ABC-method. Bar = 200 μ m. (Inset) The staining is confined to single rod shaped intracellular bacteria. ABC-method. Bar = 30 μ m.
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