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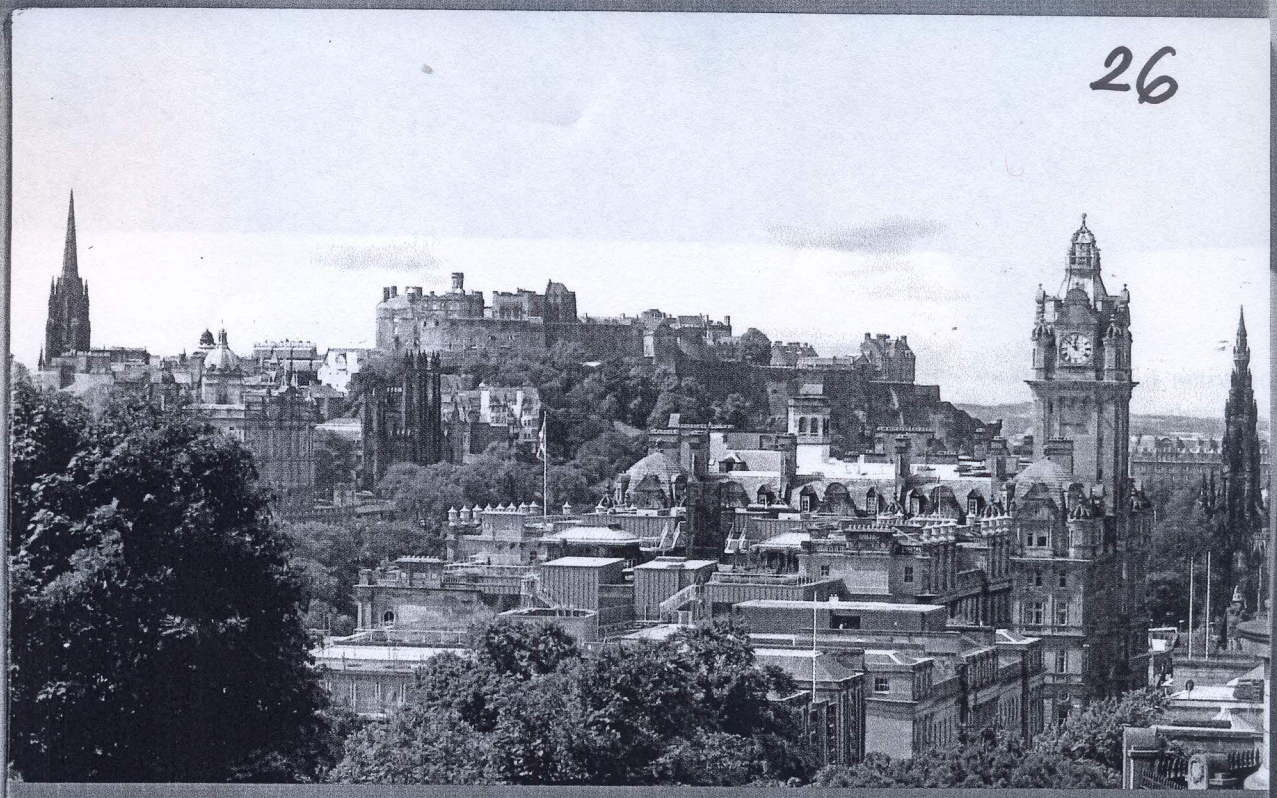

Diagnosis, Pathogenesis and Control of Animal Chlamydioses

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Detection of *Chlamydophila psittaci* in free-living birds using ELISA and immunohistochemical methods

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Chlamydophila psittaci (*C. psittaci*) is an important etiological agent for bird respiratory diseases known to be transmissible to humans causing significant infection. As an intracellular and zoonotic agent it represents difficult and hazardous material for handling. This was the main reason to perform the preliminary epidemiological investigation by antibody detection.

Because of the reported high seroprevalence rates in pigeons, in this study we wanted to determine what the infection rate was, based on *Chlamydophila* antigen detection. We also aimed to make a comparison between the sensitivity of ELISA and IHC diagnostics methods.

The total number of investigated birds using ELISA antigen detection test was 125, of which 104 were feral pigeons (*Columbia livia*), 4 were Collared doves (*Streptoelia decaocto*) and 17 different psittacine birds. Out of 104 feral pigeons 20 were positive (19.2%), 7 equivocal (6.7 %) and 77 negative (74.0 %) by ELISA. Four Collared doves (*Streptoelia decaocto*) were positive and of 17 different psittacine birds, 8 (47%) were positive, 4 (23.5%) equivocal and 5 (29.4%) negative. Post-mortem findings were characterised by swelling and fragility of the liver and spleen with greyish-white foci of necrosis.

Formalin-fixed, paraffin-embedded livers and spleens from 16 different pigeons from different towns from Macedonia were investigated using an immunohistochemical method.

Histological sections of all selected cases were stained with haematoxylin and eosin (HE) and reviewed histologically to verify the presence of chlamydial inclusions.

Histopathological findings were characterised by necrotic lesions of the liver and spleen. There were small to relatively large necrotic foci in the hepatic cells of the liver. Also, proliferated and swollen Kupffer cells were filled with debris and diffuse dilatation of sinusoids with infiltration of mononuclear cells, lymphocytes and heterophils was seen.

In the spleen, cellular proliferation and necrosis causing an enlarged appearance, infiltration and increase of macrophages associated with swelling

and activation of reticuloendothelial cells around the capsule were observed together with various foci of necrosis.

In the present study we used an immunohistochemistry method to detect chlamydial antigen in livers and spleens obtained from the sacrificed birds. Paraffin sections were investigated for the presence of chlamydial antigen using *Chlamydiaceae* family-specific mouse monoclonal antibodies and the En Vision Kit (Dako ChemMate, Glostrup, Danemark) according to the manufacturer's instructions.

From 16 cases investigated by the immunohistochemical method, 10 were positive and 6 negative. The positive reaction was in both liver and spleen sections and provided that spleens showed strong reaction with great number of positive cells. In most cases in spleens subcapsular distribution of positive cells and subcapsular dilation of blood vessels were founded.