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Short communication

Hemorrhagic pneumonia in neonatal minks in Greece concomitant with *Leishmania infantum* detection

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Abstract

In the present study a severe outbreak of hemorrhagic pneumonia (HP) in neonatal minks concomitant with *Leishmania infantum* (*L. infantum*) detection is reported. The outbreak took place on a Greek mink farm and affected 1,362 mink kits, with 524 dying. Macroscopic lesions of 14 necropsied affected kits were confined to the respiratory system with dark red, consolidated lung lobes and to the small intestine with severe, acute, hemorrhagic and necrotic enteritis. Microscopic examination of lung sections revealed severe hemorrhagic pyogranulomatous pneumonia. Bacteria were obtained in pure culture from the lungs of all necropsied animals and were confirmed as *Pseudomonas aeruginosa* (*P. aeruginosa*). Three out of 14 (21.4%) animals were positive for the presence of *L. infantum* DNA. The outbreak was attributed to the infection of minks with *P. aeruginosa*, possibly as a consequence of being immuno-suppressed by *L. infantum*. Further research is necessary, especially on the pathogenesis of *P. aeruginosa*/*L. infantum* co-infection and the implications of this interaction on HP disease outcome.

Key words: mink, hemorrhagic pneumonia, *Pseudomonas aeruginosa*, *Leishmania infantum*, Greece

Introduction

Hemorrhagic pneumonia (HP) in mink was considered as an acute and fatal disease caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) (Knox B 1953). Furthermore studies reported that clinical outbreaks of this type of pneumonic disease in farmed mink could also be associated with hemolytic strains of *Escherichia coli* (*E. coli*) (Salomonsen et al. 2013). So far HP research has considered only a single or a limited number of parameters. There is thus an urgent need to conduct more ambitious researches especially on co-infections of *P. aeruginosa*/*E. coli* in minks in different environments.

Leishmania infantum (*L. infantum*) is the etiologic agent of visceral leishmaniasis (VL) a chronic infectious zoonosis that is endemic in Mediterranean Countries. *Leishmania infantum* kinetoplast DNA was recently detected by molecular methods in an increasing number of wild animals including minks (Del Río et al. 2014) by PCR and DNA sequencing. The region is at the northern periphery of *Leishmania infantum* endemic Iberian Peninsula and infection in the dog (reservoir). However, little is known about the clinical manifestation of *Leishmania* infections in mustelidae.

Studies suggest that very complex mechanisms involving dysregulation of host immune responses contribute to *Leishmania*-mediated immune activation and pathogenesis of different infectious diseases. In respect to this, we herein describe a severe outbreak of HP in neonatal farmed minks in Greece concomitant with *L. infantum* detection.

Materials and Methods

In April 2015 diseased neonatal minks for gross pathological, histopathological and microbiological examination were collected from a farm located in Greece. The farm housed 2,000 dams and 9,200 kits in wire pens with nest boxes that were elevated off the ground. The farming conditions were excellent. Selection of the farm for further studies was done based on the clinical course that suggested HP occurrence. Fourteen diseased kits were euthanized and a necropsy was performed within the hour. Specimens of the lungs from each kit were fixed in 10% neutral buffered formalin, embedded in paraffin wax, and 5- μ m-thick sections prepared by microtome and subsequently stained with hematoxylin and eosin. In parallel, samples of lungs were cultured on MacConkey agar (CM0507, Oxoid Limited Wade Road, Basingstoke Hants, RG248PW, England) aerobically at 37°C, and on Columbia agar plus 5% sheep blood (ref. 43041, bioMérieux Marcy l'Etoile b376, chemin de l'Orme,

69280 Marcy l'Etoile), aerobically and in an atmosphere that contained 5% CO₂ at 37°C. Fourteen non-lactose fermented bacterial colonies, one per animal, were randomly selected for the following analysis: oxidase test, and species identification with polymerase chain reaction (Gholami et al. 2016). Moreover, total genomic DNA was extracted from the brain, liver, and spleen samples using a commercially available DNA extraction kit (NucleoBond PC100 kit, Macherey-Nagel GmbH Co KG, Düren, Germany) according to the manufacturer's protocol. The DNA samples were analyzed by ITS-1-nested PCR for *L. infantum* detection (Leite et al. 2010) and by a semi-nested PCR for *Toxoplasma gondii* detection (Zheng et al. 2016)

Results and Discussion

The 14 necropsied animals shared the same macroscopic lesions and histological findings. Gross lesions were confined to the respiratory system and to the small intestine. The lung lobes were dark red in color and consolidated. Severe, acute, hemorrhagic and necrotic enteritis was prominent upon opening the carcass. Microscopic examination revealed severe acute hemorrhagic pyogranulomatous interstitial pneumonia which was characterized by a thickening of the alveolar walls due to inflammatory infiltration composed of neutrophils, macrophages and lesser numbers of lymphocytes, accompanied by hemorrhages, alveolar edema and sporadic perivascular lymphocytic aggregates (Fig.1). After 48 hours of incubation, non-lactose fermented and oxidase positive colonies were obtained in pure culture from all the specimens cultured on MacConkey agar. Fourteen bacterial isolates (one per animal), were identified by PCR as *P. aeruginosa*. Three out of 14 (21.4%) animals were positive for the presence of *L. infantum* DNA. In contrast, *T. gondii* DNA was not detected in any of the examined samples.

Clinical problems at the farm started to emerge once the kits were 7 to 14 days old. The clinical course was identified as detection of dead animals, in most of the cases with blood around nostrils and mouth. Overall, the disease occurred in 1,362 (14.8%) kits; 524 (38.5%) of them died over a 2-week period. In contrast dams were unaffected and showed no signs of the disease. Undoubtedly the results confirmed HP diagnosis due to *P. aeruginosa*. Although the dams on the farm had been vaccinated against *P. aeruginosa*, maternal antibodies obviously did not offer enough protection to the kits. With respect to this, the failure of the routine vaccination as well as the severity of the pneumonic disease might be attributed to immunosuppression caused by *L. infantum*.

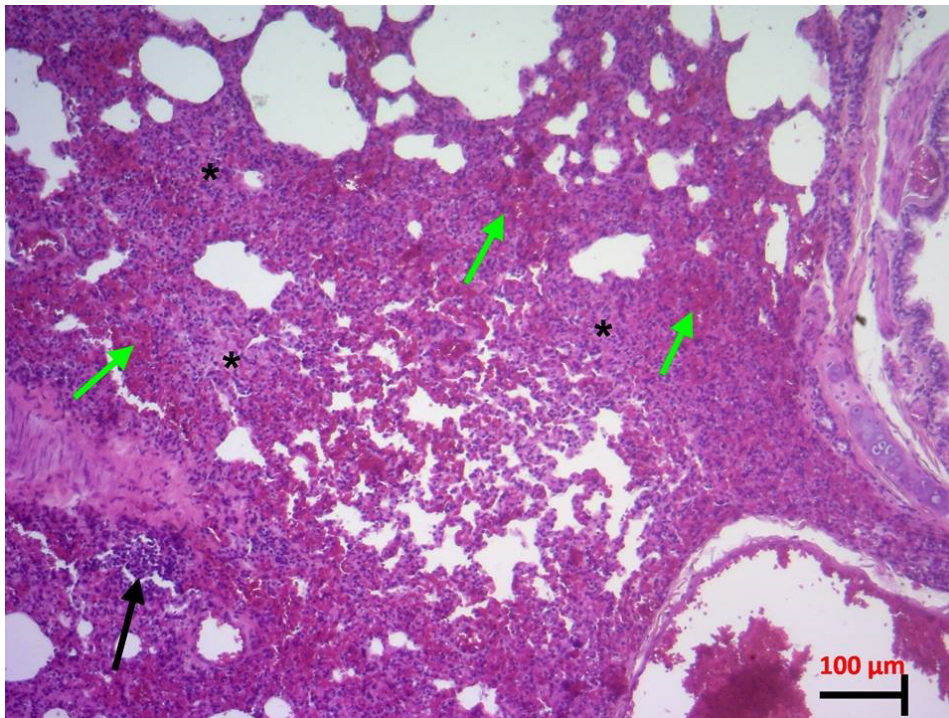


Fig. 1. Microscopic examination of hematoxylin and eosin-stained lung sections revealed severe acute hemorrhagic pyogranulomatous interstitial pneumonia which was characterized by a thickening of the alveolar walls due to inflammatory infiltration composed of neutrophils, macrophages and lesser numbers of lymphocytes (asterisks), accompanied by hemorrhages (green arrows), alveolar edema and sporadic perivascular lymphocytic aggregates (black arrow).

Whether outbreaks of HP on mink farms are related to other infectious factors has not been widely investigated. Long and Gorham (1980) focal necrosis, and widespread inflammation and necrosis. Secondary lesions of peracute hemorrhage and necrosis were the result of bacterial spread via the airways. Invasion of vessel walls by *P. aeruginosa* was a terminal event and was secondary to bacillary invasion and necrosis of adjacent tissues. Regional (lymphatic reported more serious lesions of HP in mink that were co infected with Aleutian disease virus (ADV). Secondary lesions of peracute hemorrhage and necrosis were the result of bacterial spread via the airways. Invasion of vessel walls by *P. aeruginosa* was a terminal event and was secondary to bacillary invasion and necrosis of adjacent tissues. Additionally Honda et al. (1977) described a statistically significant relation among the highest mortality occurring on the farm and the highest prevalence of antibodies against ADV. It is well documented that like ADV, *Leishmania* also modulates host immunity in ways that may affect the detection, pathogenesis and prognosis of other infectious agents (Ezra et al. 2010). Based on the results of these previous studies we conclude that further research is necessary especially on the pathogenesis of *P. aeruginosa*/*L. infantum* co-infection and the implications of this interaction on HP disease outcome.

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