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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Morphology and Morphometry of the Lung in Corn Snakes (*Pantherophis guttatus*) Infected with Three Different Strains of Ferlavirus

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Summary

Ophidian paramyxovirus (ferlavirus) is a global threat to reptilian sauropsids in herpetological collections, with occasional but fatal effects. This study characterizes the effects of three different genetic strains of ferlavirus on the dynamic changes of histology and morphometry of the lung of corn snakes (*Pantherophis guttatus*). Lungs from 42 corn snakes were either sham-infected or infected experimentally under standardized conditions. From 4 to 49 days after intratracheal inoculation, the lungs were examined qualitatively and quantitatively. Progressive microscopical changes were seen in the lung. Initially, increased numbers of heterophils were observed in the interstitium followed by proliferation and vacuolation of epithelial cells lining faveoli. Electron microscopy revealed loss of type-I pneumocytes, hyperplasia of type-II pneumocytes, and interstitial infiltrates of heterophils and mononuclear cells. With progression of disease the respiratory epithelium was initially overgrown by transformed type-II pneumocytes and later became multilayered. The results of the study suggest that the respiratory capacity of the lungs declines with disease development. The dynamics of disease development and histopathology differed in snakes infected with different ferlavirus genogroups. Animals infected with virus genogroup B developed histopathological changes and morphometric changes more rapidly and of greater intensity than snakes infected with viruses from genogroups A or C.

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Introduction

Ferlavirus belongs to the family Paramyxoviridae (Hyndman *et al.*, 2013; ICTV, 2015; Afonso *et al.*, 2016) and infects snakes primarily. Ferlavirus causes a range of clinical signs in affected snakes, including stomatitis, open-mouthed breathing, nasal discharge and purulent tracheal secretions. Neurological signs such as apathy and a straight body position may occur later in the course of disease. Snakes often die after an extended period of illness. Histopathological findings in the lungs include interstitial infiltration with heterophils and proliferation of the lining cells of the pulmonary epithelium. No specific treatment is known (Jacobson *et al.*, 1992, 1997; Schumacher, 2011). Since the first recorded outbreak of ophidian paramyxovirus (ferlavirus) in the 1970s, the virus has spread globally through herpetological collections with occasional but fatal effects on private and zoological collections. Details of the virology and immunological response of snakes to different strains of ferlavirus are reported (Neul *et al.*, 2016; Pees *et al.*, 2016).

The aims of this study were: (1) to characterize the effects of three different genetic strains of ferlavirus on the dynamic changes in histology and morphometry

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of the lung of corn snakes (*Pantherophis guttatus*), and (2) to understand quantitative changes in the respiratory epithelial surface in the lung of infected corn snakes (i.e. how and to which degree the histopathological processes successively affected oxygen exchange and therefore potentially reduced respiratory capacity). The latter question is important because the respiratory tract of snakes has a relatively large overcapacity for oxygen exchange (Starck *et al.*, 2012, 2015). Under standard conditions, the lung of snakes has a higher capacity for oxygen exchange than is required by standard metabolic rate. This overcapacity is occasionally utilized when snakes digest large prey (i.e. specific dynamic action) and oxygen consumption peaks for several days at about 10-times standard oxygen consumption (McCue, 2006; Secor, 2008). A potentially detrimental effect of this pulmonary overcapacity is that respiratory infections can spread progressively through the lung, thereby continuously reducing respiratory gas exchange without causing clinical signs. Clinical signs may develop only when oxygen exchange capacity falls below the requirements of standard metabolic rate (Starck *et al.*, 2015). This is particularly relevant when there is hyperplasia of cells of the pulmonary epithelium.

Materials and Methods

This study was part of a larger experiment that aimed to record and analyze the pathogenesis of ferlavirus infection and to assess different methods for detecting the virus. Details of the infection experiments, including a genotypic characterization of the three virus lines, are given in Pees *et al.* (2016). Details of the stereological methodology are given in Starck *et al.* (2012, 2015).

Thirty-nine adult corn snakes were purchased from a commercial company. All were checked for health status, including examination for ecto- and endoparasites as well as bacterial and fungal pathogens (by taking swabs from the choana and cloaca and a tracheal wash sample). A combined tracheal wash and cloacal swab sample was checked for the presence of ferlaviruses following an established protocol (Ahne *et al.*, 1999). Only healthy snakes were included in the study and were sorted randomly into three trial groups with 12 snakes in each, and a control group (i.e. sham-infected) of three snakes (Table 1). The snakes were housed under standardized conditions (terraria measuring 140 × 78 × 65 cm, six animals each, temperature 20–32°C with hotspots at 35°C, relative humidity 40–70 %, ground material turf, water basin and hiding places provided). The experimental design was approved by the institutional ethical committee (trial permit number TVV 61/13).

Table 1
Sample size, sex, average body length and body mass of corn snakes in the study

Group	*Male/female*	*Length (cm)*	*Body mass (g)*
A	7/5	126 (119–141)	358 (219–519)
B	6/6	121 (104–140)	350 (232–498)
C	4/8	123 (106–150)	451 (288–667)
Sham-infected	2/1	130 (126–133)	490 (410–568)

The experimental design is described in detail in Pees *et al.* (2016). Briefly, each group was allowed to acclimatize for a period of 6 days, followed by intratracheal inoculation with virus or sterile sham infection. Snakes were observed for up to 49 days post infection. Three different virus strains (genogroup A isolate 'Xeno-USA99', genogroup B isolate 'Crot-GER03' and genogroup C isolate 'PanGut-GER09') were selected (Marschang *et al.*, 2009; Abbas *et al.*, 2011). Trial groups A, B and C had infections with genogroups A, B and C, respectively. Sham infections were conducted in an identical manner except that the inoculum was prepared from sterile supernatant from virus-free cell cultures.

After infection, sampling and post-mortem examinations were conducted following a strict protocol (Pees *et al.*, 2016). On days 4, 16, 27 and 49, three snakes from each infection group were killed and examined pathologically. The three sham-infected snakes were killed and examined on day 49 after 'post' to be consistent inoculation. Corn snakes have a vestigial left lung and a well-developed, functional right lung (Wallach, 1998) as is characteristic for Colubrids (Kardong, 1972). Tissue samples were taken from the cranial (25% of the total length of the lung), middle (50% of the total length of the lung) and caudal positions (75% of the total length of the lung) of the right lung. Tissue samples were taken with random orientation. Because the caudal part of the lungs (air sac) did not contain respiratory tissue, only tissue samples from the proximal (25%) and middle parts (50%) of the right lungs were used for stereological measurements.

Light Microscopy

Samples were fixed immediately in 5% paraformaldehyde in 0.1 mol/l phosphate buffered saline (pH 7.4) for at least 48 h, then washed in buffer, dehydrated through a graded series of ethanols (up to 96% ethanol) and embedded in hydroxyethyl methacrylate (Historesin; Leica Microsystems, Wetzlar, Germany). Sections (2 μm) cut on a Microm HM340E microtome (Thermo Fisher Scientific, Waltham,

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