



# A unique novel reptilian paramyxovirus, four adenovirus types and a reovirus identified in a concurrent infection of a corn snake (*Pantherophis guttatus*) collection in Germany

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## ABSTRACT

In 2009, 26 clinical samples (organs and oral/cloacal swabs) from a total of 24 corn snakes (*Pantherophis guttatus*) from a single owner were sent to our laboratory to be tested for the presence of viruses. Paramyxoviruses (PMV), adenoviruses (AdV) and reoviruses were detected by RT-PCR, PCR and virus isolation methods. Three snakes were infected with all three viruses at the same time, while two other snakes had a double infection (PMV and reo, AdV and reo) and nine other snakes had a single infection with any of the three viruses. No viruses were detected in 10 animals. All isolated reoviruses were identical to one another and to the reptilian orthoreovirus isolate 55-02 in the partial RNA dependent RNA polymerase (RDRP) gene sequence. AdV partial polymerase sequences represented four different types, one of which was first described here: most similar to SnAdV-1, while the other three were identical to known types: SnAV-1, -2 and -3. However, the detected single PMV differed distinctly from described reptile PMV and was a new type. According to partial L gene, HN gene and U gene sequences it may be the first described representative of a third squamamid PMV cluster: "group C" within the proposed reptilian PMV genus "*Ferlavirus*". Nucleotide identity values for the L gene of the new PMV compared to group A viruses range between 76.5 and 80.3%, and between 80.5 and 81.2% compared to group B viruses. For the HN gene, these values were similar: 78.2–80% (A) and 79.9–80.5% (B) and somewhat lower for the U gene: 72.7–75.4% (A) and 69.7–70% (B). No reports on the prevalence of concurrent viral infection in captive snake populations have been published so far. The possibility of concurrent infection with several different viruses and subsequent consequences for animal health should be kept in mind when testing reptile samples for viruses.

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## 1. Introduction

Paramyxoviruses (PMV) have been isolated from several reptiles, mainly snakes, and have been character-

ized morphologically, immunologically and/or molecular biologically (Blahak, 1995; Richter et al., 1996; Ahne et al., 1999; Franke et al., 2001; Kindermann et al., 2001; Marschang et al., 2009).

The first recorded outbreak of PMV occurred in 1972 in a snake farm in Switzerland in common lancehead vipers (*Bothrops atrox*) (Fölsch and Leloup, 1976). The causative agent was named Fer de Lance virus (FDLV) and is regarded as the type species for reptilian PMV (Clark et al., 1979). Recently, the complete genome of FDLV has been fully sequenced (Kurath et al., 2004). This genome has a unique

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gene (U gene) that is absent in other PMVs, which encodes a short protein of unknown function. This feature together with the results of phylogenetic calculations supports the classification of FDLV in a new proposed genus “*Ferlavirus*” of the *Paramyxovirinae* subfamily. PMV have been detected in many different snake species from all major families including Boidae, Elapidae, Colubridae, and Viperidae. These viruses were first named ophidian (snake) paramyxoviruses (OPMV) (Essbauer and Ahne, 2001), but have since also been described in lizards and tortoises (Marschang et al., 2009; Papp et al., accepted for publication). Reptilian PMV are considered one of the most important pathogens of snakes and have been isolated from both private and zoological collections (Jacobson, 2007). They have also been described as emerging infectious diseases which may endanger wildlife (Daszak et al., 2000; Jacobson, 1993; Azevedo et al., 2001). Clinical signs observed in PMV infected snakes most commonly involve the respiratory tract, but central nervous system (CNS) disease is also regularly observed (Jacobson, 2007). Phylogenetic analysis of partial gene sequences of PMV isolated from snakes but also from lizards and a tortoise revealed that there are at least two squamamid lineages (groups) both containing snake and lizard isolates, whereas the sole tortoise PMV isolate from a Hermann's tortoise (*Testudo hermanni*) clusters as an ancient, separate lineage of the proposed new reptilian PMV genus “*Ferlavirus*” (Marschang et al., 2009). An additional study detected a squamamid PMV in a diseased leopard tortoise (Papp et al., accepted for publication), demonstrating the wide host range of some of these viruses.

Reovirus infections have been reported in several species of reptiles including snakes (Ahne et al., 1987; Blahak and Göbel, 1991; Blahak et al., 1995; Jacobson, 1986), lizards (Ahne et al., 1987; Drury et al., 2002; Marschang et al., 2002; Marschang and Papp, 2010), and tortoises (Drury et al., 2002; Marschang and Chitty, 2004). Clinical signs associated with reovirus infections have been pneumonia and neurologic problems that may appear similar to PMV infections (Wellehan et al., 2009).

The number of reports of adenovirus (AdV) infections in reptiles is increasing, apparently due to the growing popularity of reptiles as pets (Essbauer and Ahne, 2001) and to sensitive diagnostic methods (Wellehan et al., 2004). AdV infections have been reported in several reptile species including crocodiles, snakes, and lizards (Frye et al., 1994; Jacobson and Gardiner, 1990; Kinsel et al., 1997; Ramis et al., 2000). In these species, adenovirus infection was considered the cause of gastroenteritis, hepatitis, nephritis, pneumonia and encephalitis (Frye et al., 1994; Heldstab and Bestetti, 1984; Schumacher et al., 1994). The single fully sequenced reptilian isolate is from a corn snake (*Pantherophis guttatus*) (Farkas et al., 2002, 2008). This snake adenovirus type 1 (SnAdV-1) represents the supposed reptilian lineage of the *Atadenovirus* genus. Further squamamid AdVs (including new snake types) have been partially sequenced and found to belong to the same genus (Wellehan et al., 2004; Garner et al., 2008; Papp et al., 2009a; Benkő et al., 2006; Péntzes et al., 2010). Interestingly, unlike PMVs, the recently described chelonian AdVs cluster separate from the squamamid AdVs, either to the supposed amphibian lineage *Siadenovirus*

genus (Rivera et al., 2009) or outside of the five accepted genera of the family *Adenoviridae* (Farkas and Gál, 2009; Doszpoly and Harrach, personal communication; Wellehan et al., personal communication).

During routine diagnostic testing for reptilian viruses we found a concurrent ongoing infection with all three above described viruses in a collection of corn snakes including a snake that died suddenly showing dispnoe and vomitus prior to exitus. The survivors showed no specific symptoms. A follow-up study was performed in the animals from two consecutive collections for over 5 months, before they were finally dispersed. Detected viruses were partially sequence characterized, and most of the viruses were also isolated on permanent cell lines. The PMV detected in these corn snakes is new to science, and seems to be the first described representative of a third major squamamid PMV group.

## 2. Materials and methods

### 2.1. Samples

In January 2009, a dead juvenile female corn snake (*P. guttatus*) (Lab. No. 6/3/09) was dissected at the Bird and Reptile Clinic, University of Leipzig, Leipzig, Germany. Its body weight was 4 g and the head-cloaca length 29 cm. Prior to death, the snake had dyspnoea and vomitus. Gross necropsy showed cachexia. The stomach was moderately filled with brown pasty content, the gut was empty and no macroscopic lesions were observed. Histopathologically the only finding was hyperplasia of melanophagocytes in the liver, while other organs showed advanced autolysis. According to the clinical symptoms, the hyperplasia of melanophagocytes, the juvenile age and the history of another death in the snake collection, a viral disease was suspected. Samples of lung, kidney, and intestine were sent to the University of Hohenheim, Stuttgart, Germany for virus detection. One month later, 13 young corn snakes (body weight: 7–30 g) remaining in the possession of the same owners were swabbed (oral and cloacal) and tested (Lab. No. 14/1/09 to 14/13/09). Later, without further notice the owner dispersed the stock (some were reported to have died afterwards) and established a new snake collection. At this point – five months after the first tests – these 10 animals (Lab. No. 54/1/09 to 54/10/09) were also swabbed and tested. All together 26 clinical diagnostic samples including 3 organs and 23 oral and cloacal swabs from a total of 24 corn snakes of two consecutive stocks kept at the same enclosure of one owner were sent to our laboratory and tested for the presence of PMV, AdV, and reoviruses (Table 1). Tissue samples or swabs were immersed in 3 ml Dulbecco's modified Eagle's medium (DMEM) (Biochrom AG, Berlin, Germany) supplemented with antibiotics for further processing.

### 2.2. Virus isolation

Isolation of viruses was attempted from all samples on Russell's viper heart cells (VH-2, ATCC: CCL-140) and iguana heart cells (IgH2, ATCC, CCL-108). Samples in DMEM were sonicated for destruction of cells and

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