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# Paramyxoviruses in Reptiles: A Review

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## Abstract

In 1972, an outbreak of neurorespiratory disease in a Swiss serpentarium formed the basis for the first description of a paramyxovirus isolated from a reptile. In the forty years since this outbreak, there have been over 50 published reports about reptilian paramyxoviruses from all over the world. The majority of these investigations have concerned themselves with ferlaviruses (sometimes previously referred to as ophidian paramyxoviruses, or OPMV). The biology of these viruses is reviewed and this is followed by a review of the clinical findings that are associated with ferlaviral infection and the various diagnostic tests that are used to identify infected reptiles. Recently, a second, and highly divergent, reptilian paramyxovirus, Sunshine virus, was described in Australian pythons, so it is an opportune time to reflect on the paramyxoviruses that infect reptiles.

## Keywords

Reptile; snake; Ferlavirus; Sunshine virus; paramyxovirus; virus

## Introduction

A wide range of viruses have been detected in reptiles throughout the world and the interested reader is referred to the reviews by Wellehan and Johnson (2005), Jacobson (2007), Marschang (2011) and Ariel (2011) for more general overviews of these viruses. This review will focus on the paramyxovirus infections of reptiles. Since an outbreak of neurorespiratory disease in a Swiss serpentarium that was associated with a paramyxovirus (Folsch and Leloup, 1976), reptilian paramyxoviruses have been described in other regions of Europe (Ahne *et al.*, 1987; Blahak, 1995; Manvell *et al.*, 2000; Franke *et al.*, 2001), USA (Jacobson *et al.*, 1980; Jacobson *et al.*, 1981; Potgieter *et al.*, 1987; Richter *et al.*, 1996) and Brazil (Nogueira *et al.*, 2002; Kolesnikovas *et al.*, 2006). Most recently, a novel paramyxovirus, named Sunshine virus, was described in Australian pythons (Hyndman et al., 2012a; Hyndman et al., 2012b) and so it is important to review the established and emerging areas in this field.

## Taxonomy of Reptilian Paramyxoviruses

Members of the family *Paramyxoviridae* are currently divided into two subfamilies: *Pneumovirinae* and *Paramyxovirinae* (ICTV, 2013). *Paramyxovirinae* currently contains seven genera, one of which is the

genus *Ferlavirus*. Prior to the discovery of Sunshine virus, all phylogenetically-characterised reptilian paramyxoviruses have clustered within *Ferlavirus* (Marschang *et al.*, 2009). Sunshine virus clusters within *Paramyxoviridae* but outside of both subfamilies and therefore broadens our understanding of the diversity of paramyxoviruses that infect reptiles (Hyndman *et al.*, 2012a).

## Ferlavirus

The literature has not shown conformity in how it refers to ferlaviruses. Since the first characterisation of a snake paramyxovirus that was named Fer de Lance Virus (FDLV) (Clark *et al.*, 1979), the term ophidian paramyxovirus (often abbreviated to OPMV or oPMV) has also been used to describe the paramyxoviruses found in snakes (Lloyd and Flanagan, 1991; Homer *et al.*, 1995; Jacobson *et al.*, 1997; Manvell *et al.*, 2000; Kindermann *et al.*, 2001; Oros *et al.*, 2001; Nogueira *et al.*, 2002; Kolesnikovas *et al.*, 2006). In 2009, a proposal was put forward by Kurath to the International Committee on Taxonomy of Viruses (ICTV) to create the new genus *Ferlavirus* with *Fer-de-Lance paramyxovirus* (the same virus as FDLV) as its type species. This proposal has been accepted by the ICTV (2013) and all future work should refer to these viruses as ferlaviruses. By avoiding the general term “ophidian paramyxovirus”, Sunshine virus and the ferlaviruses of snakes can be unambiguously delineated.

Folsch and Leloup (1976) produced the first report of a reptilian paramyxovirus following an outbreak of neurorespiratory disease in a Swiss serpentarium. The physicochemical traits of this first isolate were then characterised and described by Clark *et al.* (1979). The origin of the ferlaviruses is unknown but one reference provides further insight. A personal communication mentioned in a paper by Kolesnikovas *et al.* (2006) states that the Brazilian lancehead vipers (*Bothrops moojeni* but incorrectly referred to as Fer-de-Lance vipers [*B.atrox*] in earlier works) in the Swiss serpentarium originated from Brazil. No further information is provided.

The entire genome of *Fer-de-Lance paramyxovirus* has been sequenced (Kurath *et al.*, 2004). The genome is 15,378 nucleotides long and is made up of seven distinct genes: 3' – Nucleocapsid (N) – Unknown (U) – Phosphoprotein/Protein V (P/V) – Matrix (M) – Fusion (F) – Haemagglutinin-Neuraminidase (HN) – RNA-Dependent RNA Polymerase (L). The fusion gene has been analysed by others (Franke *et al.*, 2006). Several authors have analysed the phylogenetic relationships that exist between ferlaviruses (Ahne *et al.*, 1999b; Franke *et al.*, 2001; Kindermann *et al.*, 2001; Marschang *et al.*, 2009; Papp *et al.*, 2010a; Papp *et al.*, 2010b; Abbas *et al.*, 2011) while others have compared the ferlaviruses to other paramyxoviruses (Junqueira de Azevedo *et al.*, 2001; Kurath *et al.*, 2004; Marschang *et al.*, 2009). These studies support the classification of the squamate ferlaviruses as a single genus

96 containing at least three distinct genogroups (A, B and C). The clinical
97 significance and serodiagnostic implications of the different genotypes
98 remains undefined.
99
100 The serological relatedness of ferlaviruses to other paramyxoviruses has
101 been reported by several authors (Clark *et al.*, 1979; Potgieter *et al.*,
102 1987; Blahak, 1995; Richter *et al.*, 1996; Ahne *et al.*, 1999b). Clark *et al.*
103 (1979) titrated antisera against 19 myxoviruses (16 paramyxoviruses
104 and 3 orthomyxoviruses) against *Fer-de-Lance paramyxovirus* and then
105 did the reverse by titrating ferlavirus antisera against the same suite of
106 myxoviruses. No cross-reactivity was detected. Richter *et al.* (1996)
107 showed that the antisera specific for eight paramyxoviruses did not
108 inhibit the haemagglutinating ability of three ferlaviral isolates. Ahne *et*
109 *al.* (1999b) was also unable to demonstrate any cross-reactivity
110 between ferlaviral antisera and a range of paramyxoviruses.
111
112 In contrast to these findings, serological relationships between
113 ferlaviruses and other paramyxoviruses have been shown by other
114 authors. Blahak (1995) demonstrated a serological relationship between
115 ferlavirus and *Avian paramyxovirus* types 1 and 7 (aPMV-1 and -7),
116 while Gravendyck *et al.* (1998) reported on the cross-reactivity of a
117 paramyxovirus from a monitor (*Varanus prasinus*) with aPMV-7. Later,
118 Manvell *et al.* (2000) classified two isolates of ferlavirus as “ophidian
119 paramyxovirus type 1 (PMV-1) and ophidian paramyxovirus type 7

(PMV-7)" based on the strength of their serological cross-reactivity with antisera against aPMV-1 and -7. In another report, Potgieter *et al.* (1987) used immunohistochemical staining to detect ferlavirus in a section of infected snake lung after the lung had been treated with the fluorescently-labelled antisera of *Parainfluenza virus* type 2.

The incongruence that exists in the conclusions of the studies on ferlaviral serological relatedness could be explained by a serological *un*relatedness between the various ferlaviral isolates used in these studies. Serological unrelatedness between ferlaviral isolates has been shown in at least two studies (Marschang *et al.*, 2002; Allender *et al.*, 2008). In general, however, it seems reasonable to conclude that the serological relatedness of the ferlaviruses to other paramyxoviruses is limited at most.

All ferlaviruses are believed to have neuraminidase activity. Using a substrate that is specifically cleaved by neuraminidase into an intensely fluorescent product (Yolken *et al.*, 1980), significant neuraminidase activity was detected in three isolates of ferlavirus (Richter *et al.*, 1996). Clark *et al.* (1979) also demonstrated the presence of neuraminidase activity in ferlavirus. After haemagglutinating chicken and guinea pig erythrocytes with ferlavirus it was observed that these erythrocytes could not be re-agglutinated by ferlavirus, implying that the virus has a receptor destroying enzyme (neuraminidase).

## *Clinical Findings Associated with Infection*

Ferlaviral infections have been associated with highly pathogenic disease outbreaks (Folsch and Leloup, 1976; Jacobson *et al.*, 1980; Jacobson *et al.*, 1981; Jacobson *et al.*, 1992; Kolesnikovas *et al.*, 2006). Infection has been detected in several snake families: Colubridae, Elapidae, Viperidae, Crotalidae, Boidae and Pythonidae (Jacobson *et al.*, 1997; Ahne *et al.*, 1999b; Oros *et al.*, 2001). One report described the clinical signs associated with ferlaviral infection as being variable, often non-specific, and occasionally subtle (Sand *et al.*, 2004). When clinical signs can be attributed to a particular organ system, they are most commonly localised to the respiratory tract (Jacobson, 2007) but there are also reports about snakes suffering from neurological disease. Table 1 outlines the clinical signs reported by various authors.

In 1991, Lloyd and Flanagan described the clinical manifestations of ferlaviral infection as fitting into three discrete clinical syndromes: snakes affected acutely or peracutely; “poor doers”; and, clinically-healthy animals that shed virus in the face of high antibody titres. These observations were based on clinical experience and were not from a controlled study.

## *Gross Pathology*

Significant changes that are seen at necropsy are often localised to the respiratory system (Table 2). It is important to note that more than one

author has not detected any gross necropsy changes in snakes that were later identified to be infected with ferlavirus.

## *Histopathology*

There are no histological signs that are pathognomonic for ferlaviral infection (Ritchie, 2006). Instead, a wide range of histopathological findings have been reported that are most commonly attributed to the respiratory and neurological systems (Table 3). Intranuclear or intracytoplasmic viral inclusions may be seen and these should heighten the pathologist's suspicions of ferlaviral infection (Jacobson, 2007). Ultrastructurally, these inclusions have been shown to consist of strands of viral nucleocapsid (Jacobson, 2007). Although less specific for ferlaviral infection than viral inclusions, proliferative pneumonia and perivascular cuffing in the brain are changes commonly reported in the literature. Jacobson *et al.* (2001) has noted that inclusion body disease (IBD), mycoplasmosis and infection with orthoreovirus form important rule-outs during an investigation of snakes affected with proliferative pneumonia.

Through the use of immunohistochemistry, Homer *et al.* (1995) was able to localise pulmonary infections to the luminal surface and cytoplasm of faveolar epithelium. With a similar purpose, Sand *et al.* (2004) used *in situ* hybridisation to locate ferlavirus in a variety of infected organs. Virus was intranuclear in the brain while being

intracytoplasmic in hepatocytes, Kupffer cells, pulmonary alveolar [faveolar] macrophages, respiratory epithelial cells and renal tubular epithelial cells.

## *Non-Ferlaviral Microbiological Findings*

A number of authors have reported on the non-ferlaviral microbiological findings in snakes infected with ferlavirus. Bacterial cultures from various organs have identified several Gram negative bacterial pathogens: *Aeromonas*, *Citrobacter*, *Escherichia*, *Enterobacter*, *Morganella*, *Proteus*, *Providencia*, *Pseudomonas*, *Salmonella* and *Serratia* (Folsch and Leloup, 1976; Jacobson et al., 1981; Blahak et al., 1991; Jacobson et al., 1992; Homer et al., 1995; Oros et al., 2001; Kolesnikovas et al., 2006; Jacobson, 2007). In one of these studies, fungal elements could not be cultured (Kolesnikovas *et al.*, 2006). It has been suggested that ferlaviral infections may be immunosuppressive, possibly due to lymphoid depletion (Oros *et al.*, 2001), allowing secondary bacterial invaders (Kolesnikovas *et al.*, 2006). So while it is recommended that concurrent complicating bacterial infections be treated as early as possible (Jacobson *et al.*, 1992; Kolesnikovas *et al.*, 2006), antibiotic use may not provide any improvement in already affected snakes (Folsch and Leloup, 1976; Jacobson *et al.*, 1981).

Papp *et al.* (2010a) has described the isolation of orthoreoviruses from various organs from four snakes that were positive by PCR for the

presence of ferlavirus. In a second study, Abbas *et al.* (2011) simultaneously detected at least one atadenovirus, an orthoreovirus and a ferlavirus in each of three corn snakes. One of these snakes, a juvenile, was vomiting and displayed dyspnoea before dying. These recent studies form the first reports of mixed viral infections in snakes infected with ferlavirus.

## *Transmission*

Little is known about ferlaviral transmission. Koch's postulates were fulfilled after the successful infection (endotracheal inoculation) and re-isolation of ferlavirus in a group of six naïve captive-bred Aruba Island rattlesnakes (*Crotalus unicolor*) (Jacobson *et al.*, 1997). Another three snakes were sham-inoculated. Ferlavirus was successfully isolated from the lungs of all the snakes that had been inoculated with virus and none of the sham-inoculated snakes.

Pasmans *et al.* (2008) states that ferlavirus is easily transmitted through both aerosols and contact, and terraria for individually housed snakes provide little defense against the transmission of ferlavirus. It has also been suggested that ferlavirus may be transmitted from snake to snake by direct contact, respiratory secretions, fomites and ectoparasites (especially mites) (Hernandez-Divers, 2006). In the first outbreak described by Folsch and Leloup (1976), the infection spread from the enclosures closest to the doors in two different rooms. It was

hypothesised that spread had occurred either by aerosol or via the keepers. Considering that ferlaviruses have been isolated from the sputum of a rattlesnake (*Crotalus durissus terrificus*) (Nogueira *et al.*, 2002), oral and cloacal swabs from corn snakes (*Pantherophis guttatus*) (Abbas *et al.*, 2011) and detected by polymerase chain reaction (PCR) in oral and cloacal swabs (Papp *et al.*, 2010a), it is reasonable to assume that ferlavirus can be transmitted between snakes by both oral secretions and cloacal excretions. To the best of our knowledge, ferlavirus has not been isolated, or detected by PCR, from fomites or ectoparasites. There are currently no reports concerning vertical transmission of ferlavirus (Pasmans et al., 2008).

The incubation period of ferlavirus in naturally-acquired infections is unknown. There are claims that the incubation period for ferlavirus may be as short as 21 days (Hernandez-Divers, 2006) but will generally exceed 90 days (Hernandez-Divers, 2006; Ritchie, 2006). These claims are not supported by controlled studies.

The shedding patterns of ferlavirus are unknown (Jacobson and Origgi, 2007). Although Lloyd and Flanagan (1991) state that some snakes are capable of shedding virus for an extended period of time, this claim is based on the observation that some snakes have significant haemagglutination inhibition (HI) antibody titres for five months or more while simultaneously “causing” seroconversion in cagemates

throughout that time. Ritchie (2006) suggests that asymptomatic seropositive snakes may be persistently-infected shedders while others may have mounted an appropriate immune response and cleared the infection. There are no controlled studies to support any of these claims.

## *Treatment*

No specific treatment has been identified as being effective against ferlavirus (Marschang and Chitty, 2004). In people, the antiviral drug ribavirin (Virazole®) is sometimes used in the treatment of *Measles virus, Respiratory syncytial cell virus* and *Parainfluenza virus* infections (Chakrabarti *et al.*, 2001; Freeman *et al.*, 2004). A drug named BCX 2798 that is capable of targeting paramyxoviral neuraminidase has been shown to decrease viral titres in mice infected with a recombinant strain of *Sendai virus* (Alymova *et al.*, 2005; Watanabe *et al.*, 2009). Neither of these compounds has been tested against ferlavirus either *in vitro* or *in vivo*.

Symptomatic treatment has generally been provided by broad spectrum antibiotics (Kolesnikovas *et al.*, 2006). Bronson and Cranfield (2006) have stated that the survival time of snakes infected with ferlavirus is improved by targeting secondary protozoal and bacterial infections.

## *Prevention*

### Quarantine

To prevent the introduction of ferlavirus into a collection it is recommended that new animals are only introduced after a period of quarantine. Recommended lengths of quarantine vary between references (Table 4) but there is a general trend where modern recommendations are for longer periods of time. All of the recommendations are empirical due to the limited information that is available about ferlaviral transmission and environmental viability.

Various authors have provided specific recommendations about caring for a collection consisting of resident and quarantined animals (Jacobson et al., 1999; Marschang and Chitty, 2004; Ritchie, 2006; Pasmans et al., 2008). During quarantine, agent-specific testing can be used to help determine if a snake has been exposed to, or is infected with, ferlavirus.

### Vaccination

There have been only two reported attempts to develop a ferlavirus vaccine. In one study, a sustained and significant concentration of circulating anti-ferlavirus antibodies could not be elicited in a group of western diamondback rattlesnakes (*Crotalus atrox*) following inoculation with an inactivated (killed) strain of ferlavirus (Jacobson *et al.*, 1991). Vaccinated snakes were not challenged with unattenuated

virus. Mayr *et al.* (2000) has suggested that cell culture-adapted ferlaviral isolates could be used in the production of live vaccines but in a brief and limited report of a vaccine trial using a modified-live isolate of ferlavirus, one snake died and another suffered severe illness (Lloyd and Flanagan, 1991).

## *Ferlavirus in Non-Captive Free-Ranging Snakes*

Only a few reports have described ferlavirus in wild snakes. A survey of ten free-ranging anacondas (*Eunectes murinus*) from Venezuela involved serological testing against a number of pathogens, including ferlavirus (Calle *et al.*, 2001). Ferlaviral-specific antibody titres were negative by haemagglutination inhibition (HI) in all snakes.

In two separate studies Allender *et al.* (2006; 2008) screened wild-caught eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) for the presence of anti-ferlavirus antibodies using HI. All 20 snakes from the 2006 study were seropositive against two ferlaviral isolates at one diagnostic laboratory. In the 2008 study, 26 snakes were tested and zero to 26 of them were seropositive depending on the isolate that was used as antigen and the laboratory that the samples were sent to. These discordant antibody results on a standardised sample set highlighted the variability that existed between diagnostic laboratories. Additional testing that is capable of detecting ferlavirus (e.g. virus isolation, polymerase chain reaction, *in situ* hybridisation or electron microscopy)

was not performed.

## *Ferlavirus in Non-Snake Reptilian Hosts*

### Lizards

Most of the paramyxoviruses that have been described in lizards have not been associated with overt disease. A paramyxovirus was isolated from a false tegu (*Callopistes maculatus*) in 1988 (Ahne and Neubert, 1991) that was later identified as a ferlavirus by sequence analysis (Ahne *et al.*, 1999b). Similarly, a paramyxovirus was isolated from the mouth of an apparently healthy monitor lizard (*Varanus prasinus*) that was part of a reptile collection that had suffered an outbreak of ferlavirus in its snake population (Gravendyck *et al.*, 1998).

In a serological survey of lizards, Gravendyck *et al.* (1998) collected serum from 49 healthy free-ranging Honduran Island iguanas (*Ctenosaura bakeri*, *C.similis* and *Iguana iguana rhinolopha*) to look for evidence of paramyxoviral and reoviral infections. Using a reptilian paramyxovirus isolated from a monitor lizard (*Varanus prasinus*) as antigen, 41% of all 49 serum samples had antibodies that could be detected by virus neutralisation. 34 serum samples were tested against this paramyxovirus isolate by haemagglutination inhibition and only three (9%) had antibody titres of ≥20 (maximum titre was 32). The authors could not isolate any viruses from pharyngeal and cloacal swabs from these lizards. In a similar study, Marschang *et al.* (2002) tested 30

wild-caught Mexican lizards (*Xenosaurus grandis*, *X.platyceps* and *Abronia graminea*) for exposure to paramyxovirus and reovirus: 30 were tested by virus isolation and 23 were tested by virus neutralisation (reovirus) and HI (paramyxovirus). Anti-ferlavirus antibodies were detected in four animals representing all three species but significantly, a ferlavirus was isolated from the cloacal swab of *X.platyceps*. The results of this report were unable to clarify the clinical significance of finding ferlavirus and ferlaviral antibodies in these species.

Lloyd *et al.* (2005) serologically tested the lizard population of a zoological park where the resident snake collection had a history of paramyxovirus-associated disease. In total, 59 lizards (from 12 families) were tested for the presence of ferlavirus-specific antibodies by haemagglutination inhibition (HI). All the lizards, except one, were clinically normal. Seven lizards had HI titres that were considered positive (≥16) for exposure to ferlavirus. These seven positive animals were then retested 105 days later and six of them had either maintained or increased their antibody titres. The authors concluded that the six animals had active infection, were repeatedly exposed to antigen or were in a carrier state. In another study, ferlavirus exposure was serologically assessed in 32 geckos (*Gecko monarchus* and *Gehyra mutilata*) that free-roamed the grounds of a zoological park (Kummrow *et al.*, 2004). Blood was pooled from these small geckos and 70% of pooled blood samples tested positive for ferlavirus exposure by HI. The

authors speculate that the geckos may have a role as vectors for this virus but more detailed investigations would be necessary to elucidate this idea further.

There are only two reports where a paramyxovirus was associated with mortality in a lizard (Jacobson *et al.*, 2001; Boyer *et al.*, 2005). Three separate epidemics of ferlavirus were seen between 1998 and 1999 in caiman lizards (*Dracena guianensis*) that had been imported into the USA from Peru (Jacobson *et al.*, 2001). Many individuals were found dead or were anorexic upon arrival. Histopathology showed severe heterophilic and histiocytic pneumonia and ferlavirus was detected in tissue sections by immunohistochemistry. A virus was isolated and electron microscopic examination confirmed the presence of a paramyxovirus. In the second report, paramyxovirus-like particles were seen by electron microscopy in the respiratory tract of a Thai water dragon (*Physignathus concinus*) (Boyer *et al.*, 2005). Histological assessment revealed a proliferative interstitial pneumonia and eosinophilic intracytoplasmic inclusions of the pneumocytes and pancreatic ductular epithelium. DNA probes could not detect a reptilian paramyxovirus in paraffin-embedded sections.

## Chelonians

In 1983, Jackson and Needham reported on the discovery of anti-Sendai virus haemagglutination inhibition (HI) titres of up to 256 in 34 tortoises

from three species: *Testudo graeca* (Greek tortoise), *T.hermanni* (Herman's tortoise) and *Geochelone elegans* (Indian star tortoise). Seven of these 34 tortoises were showing signs of rhinitis at the time of blood sampling. There was little correlation between anti-Sendai virus HI titre and the presence of clinical signs. The authors did not attempt to isolate a paramyxovirus from any of these tortoises. In another study, a collection of Mediterranean tortoises (*Testudo graeca* and *T.hermanni*) were imported from Turkey to Switzerland and upon arrival many were found to have a viral dermatitis (Zangger *et al.*, 1991). Light microscopy revealed intracytoplasmic inclusions in the stratum germinativum and under electron microscopy, paramyxovirus-like particles were identified.

In 1990, Oettle *et al.* (1990) reported on the death of 31 out of 83 African tortoises from four species: *Psammobates tentorius* (tent tortoise), *Homopus areolatus* (beaked cape tortoise), *Chersina angulata* (bowsprit tortoise) and *Geochelone pardalis* (leopard tortoise). Affected tortoises displayed a wide range of clinical signs and pathological changes but anorexia, progressive lethargy, severe ascites, hepatosis, pneumonia and necrotic pseudomembranous stomatitis were seen most commonly. Paramyxovirus-like inclusions were identified under light microscopy in oesophageal cells from one tortoise. It is likely that a range of disease processes featured in this outbreak and whether a paramyxovirus was present, and if so, the role it played, is unclear.

In 1993, Witte found three out of 128 tortoises from ten different collections revealed antibody titres against a snake paramyxovirus. Titres were 1:16 in two Russian tortoises (*Agrionemys* [*Testudo*] *horsfieldii*) and 1:32 in a Greek tortoise (*Testudo graeca*).

In 1999, a ferlavirus was isolated from a Hermann's tortoise (*Testudo hermanni*) suffering from pneumonia (Marschang *et al.*, 2009). The identity of this ferlavirus was confirmed by sequence analysis. More recently, several different ferlaviruses were detected by PCR from various organs of a leopard tortoise (*Geochelone pardalis babcocki*) suffering from respiratory distress (Papp *et al.*, 2010b). There were large amounts of mucopurulent discharge from its nares and mouth and on necropsy, the lungs were bilaterally consolidated and filled with thick serous exudate. The ferlaviruses detected by PCR could not be isolated into cell culture.

In the most recent study on ferlaviruses in tortoises, tortoise plasma was screened for antibodies against ferlaviruses using haemagglutination inhibition testing. Antibodies were found in several tortoise species from several European countries (Rösler *et al.*, 2013).

## Crocodiles

Using electron microscopy, paramyxoviruses have been found in the faeces of Nile crocodiles (*Crocodylus niloticus*) that had been fed

chickens from a farm that were having an outbreak of *Newcastle disease virus* (Huchzermeyer *et al.*, 1994). This study also provided limited information about a paramyxovirus that was seen in the faeces of a crocodile not fed a diet of chickens.

Only a small proportion of the reports about ferlavirus in non-snake reptiles show a strong association between disease and infection with this pathogen but there is insufficient data to ignore the possibility that lizards, chelonians and maybe even crocodiles, play important roles as reservoir hosts for ferlavirus. This could have important implications when attempting to eradicate ferlavirus or prevent its introduction in collections that house snakes with other reptiles.

## *Zoonotic Potential*

Ahne and Mayr (2000) investigated the capability of ferlavirus to infect human blood mononuclear cell culture at the virus-permissive temperature of $28^0$C. Viral replication could not be detected in this cell line. Potgieter *et al.* (1987) successfully cultured a paramyxoviral isolate from a snake in hamster kidney cells at $37^0$C but this study found that the highest haemagglutination titre, the greatest likelihood to grow in cell culture and the most significant cytopathic effects were seen when the isolate had been grown at $30^0$C. Under the conditions described by other authors, it was found that ferlavirus did not replicate at $37^0$C (Clark et al., 1979; Blahak, 1995; Ahne et al., 1999a; Ahne and Mayr,

2000). Clark *et al.* (1979) comprehensively investigated the susceptibility of mice to infection with ferlavirus and could not detect any clinical or histological evidence of disease. Based on this information it would seem unlikely that ferlavirus would pose a serious zoonotic risk to human health.

## *Diagnostic Tests*

### Virus Isolation

Tissue samples and oral and cloacal swabs are often used to isolate viruses from infected reptiles (Marschang and Chitty, 2004). Table 5 provides a summary of the techniques that have been used to isolate paramyxoviruses from reptiles. The cytopathic effect associated with infection from reptilian paramyxoviruses has been described by a number of authors (Table 6). Syncytial cell formation and cell lysis are commonly reported. The time taken for CPE to emerge varies markedly between references: from only 24 to 36 hours (Ahne *et al.*, 1987) to requiring serial passage (Jacobson *et al.*, 1980). The successful isolation and/or propagation of reptilian paramyxoviruses using embryonated eggs have been reported (Clark *et al.*, 1979; Ahne *et al.*, 1999a). Similar attempts by other authors were unsuccessful (Potgieter *et al.*, 1987; Manvell *et al.*, 2000) (Table 5).

### Electron Microscopy

Many authors have utilised transmission electron microscopy (TEM) to

identify reptilian paramyxoviruses (Lunger and Clark, 1978; Clark *et al.*, 1979; Jacobson *et al.*, 1980; Jacobson *et al.*, 1981; Ahne *et al.*, 1987; Potgieter *et al.*, 1987; Richter *et al.*, 1996; Jacobson *et al.*, 1997; Manvell *et al.*, 2000; Franke *et al.*, 2001; Jacobson *et al.*, 2001; West *et al.*, 2001). Ferlaviruses are medium-sized, have spiked envelopes and can be spherical to pleomorphic in morphology (Jacobson and Samuelson, 2007). The nucleocapsid of a paramyxovirus forms the core of the virion and has a distinct "herring bone" appearance (Ahne and Mayr, 2000). Spherical and filamentous forms of ferlavirus have been seen budding from infected cells (Jacobson *et al.*, 1997).

Inclusion bodies have been identified under light microscopy in snake tissue infected with ferlavirus (Jacobson *et al.*, 1981; Potgieter *et al.*, 1987; Homer *et al.*, 1995; Jacobson *et al.*, 1997; West *et al.*, 2001). Ultrastructural assessment has shown these inclusions to be comprised of nucleocapsid strands (Jacobson *et al.*, 1981; Jacobson *et al.*, 1997).

## Haemagglutination (HA) Assays

Large quantities of virus are needed for macroscopic haemagglutination so this method is considered to be relatively insensitive (Quinn *et al.*, 2002). In one study, ferlavirus-infected tissue homogenates did not haemagglutinate chicken erythrocytes but ferlavirus could be isolated onto viper heart cells and be detected by polymerase chain reaction (Kolesnikovas *et al.*, 2006). Only after replication in viper heart cells,

could haemagglutination be detected. Studies comparing the lower limits of detection of haemagglutination to other diagnostic tests, such as polymerase chain reaction, do not exist.

## Haemagglutination Inhibition (HI)

Haemagglutination inhibition (HI) has been used widely as a serological test for the detection of exposure to ferlavirus (Jacobson *et al.*, 1981; Potgieter *et al.*, 1987; Jacobson *et al.*, 1991; Jacobson *et al.*, 1992; Brousset *et al.*, 1994; Blahak, 1995; Richter *et al.*, 1996; Jacobson *et al.*, 1997; Gravendyck *et al.*, 1998; Manvell *et al.*, 2000; Calle *et al.*, 2001; Jacobson *et al.*, 2001; Marschang *et al.*, 2002; Lloyd *et al.*, 2005; Allender *et al.*, 2006; Allender *et al.*, 2008) and is offered commercially by several diagnostic laboratories (Table 7).

Various vertebrate erythrocytes have been compared to each other in their ability to haemagglutinate three isolates of ferlavirus (Richter *et al.*, 1996). Chicken and guinea pig erythrocytes reliably haemagglutinated these three isolates and outperformed sheep, human type-O and rabbit erythrocytes. Many, if not all, of the laboratories that offer HI commercially utilise chicken or guinea pig erythrocytes as markers of ferlavirus-induced haemagglutination (Allender *et al.*, 2008).

There is disagreement in the literature about the titres that should be

534 considered positive for exposure to ferlavirus. Titres of greater than 10
535 (Jacobson *et al.*, 1992) and 16 (Pasmans et al., 2008) have been
536 reported but it has also been suggested that less than 20 is negative,
537 between 40 and 80 is suspect and greater than 80 is positive (Jacobson
538 and Origgi, 2007).
539
540 Pasmans *et al.* (2008) recommends that paired samples, eight weeks
541 apart, be taken to determine if a snake has a rising antibody titre. A
542 rising titre may indicate *current* exposure to ferlavirus, while a
543 "positive" titre that does not increase (i.e. stays the same or decreases)
544 may be indicative of *previous* exposure (Jacobson and Origgi, 2007). HI
545 assays quantify the ability of serum or plasma to inhibit macroscopic
546 haemagglutination without delineating the contributions to this
547 inhibition that were made by immunoglobulin M (IgM) and IgY (the
548 reptilian equivalent of IgG). In addition to this, controlled experiments
549 that were able to identify the antibody titres at several time points
550 during and after antigen exposure do not exist. So conclusions drawn
551 from rising, falling or unchanged anti-ferlavirus HI titres may not always
552 be reliable. Despite these limitations, reports do exist that provide
553 useful information about the HI titres that were seen during a
554 controlled transmission study and also naturally occurring outbreaks of
555 ferlavirus infection.
556
557 An experimental transmission of ferlavirus in Aruba Island rattlesnakes

(*Crotalus unicolor*) forms the only study where ferlavirus was inoculated into snakes under controlled conditions (Jacobson *et al.*, 1997). In this study, HI titres were only assessed at the time of death. The last death occurred 22 days after inoculation. No snake had developed an antibody response that could be detected by HI.

In an outbreak of ferlavirus in a zoological collection, Jacobson *et al.* (1992) tested 31 snakes for the presence of anti-ferlavirus antibodies by HI. Twelve snakes showed positive titres (greater than 10) and these animals were then retested a number of times over the next year. Many cases showed high titres (5,120 to greater than 20,480) that decreased to low titres (below 100) over three to seven months.

In another outbreak of ferlavirus in reptiles, this time a collection of caiman lizards (*Draecena guianensis*), HI testing was performed on surviving animals several months after a ferlavirus had been isolated from dead animals (Jacobson *et al.*, 2001). From 17 animals tested, there were seven titres of less than or equal to 20 and ten titres were between 20 and 180.

An HI titre is complicated by a long list of variables: the antibody's affinity to the antigen, the integrity of the antigen being used, the availability of antibody in the serum, the preservation of the sample and lastly, the host's immune response, which itself is influenced by

582 temperature, the season, nutritional status, antigen concentration,
583 route of inoculation, frequency of exposure to the antigen and the type
584 of antigen (Lloyd *et al.*, 2005). The influences that these factors have on
585 an HI titre have not been investigated in a controlled experiment and so
586 the consideration that should be made to each of these factors can only
587 be speculated. Some zoological collections and private institutions
588 require negative ferlavirus titres during quarantine before a snake is
589 released into the main collection (Allender *et al.*, 2008) and the
590 difficulties in interpreting HI titres places the decision-making
591 veterinarian in a difficult position.
592
593 According to Lloyd *et al.* (2005), if there is a serological unrelatedness
594 between the ferlavirus that has been used as antigen in an HI assay and
595 the ferlavirus the animal has been exposed to, negative HI titres may
596 occur. For this reason, other authors have recommended that two
597 different viral isolates are used as the antigen source to accommodate
598 serological differences that might exist between ferlavirus strains
599 (Pasmans et al., 2008). In a study of 60 snake serum samples that were
600 being tested by HI using two different strains of ferlavirus as antigens,
601 considerable variation in HI titre was seen between the two antigens
602 but most snakes that were considered to be positive, were positive
603 using either antigen (Kania *et al.*, 2000).
604
605 In contrast to the findings of Kania *et al.* (2000), Allender *et al.* (2008)

found there was considerable variation in the HI titres of 26 wild-caught eastern massasaugas (*Sistrurus catenatus catenatus*) when analysed at three American commercial laboratories, which between them, utilise four different isolates of ferlavirus as antigen. Against two antigens, 100 percent of plasma samples were positive, 56 percent were positive against the third and none were positive against the last. The diagnostic implications of these results are unknown but making decisions based on HI serology may be problematic.

## Non-haemagglutination Inhibition Antibody Assays

Only two reports describe the detection of anti-ferlaviral antibodies using non-haemagglutination inhibition assays. Serum neutralisation was used by Gravendyck *et al.* (1998) to detect antibodies against a reptilian paramyxovirus (isolated from a monitor lizard, *Varanus prasinus*) in 49 free-ranging Honduran Island iguanas (*Ctenosaura bakeri*, *C.similis* and *Iguana iguana rhinolopha*). It was found that 41% of the serum samples had antibodies that could be detected by virus neutralisation. This compares to 9% (out of a subset of 34 animals) that had haemagglutination inhibition (HI) antibody titres of ≥20 (maximum titre was 32). In the second study, an enzyme-linked immunosorbent assay (ELISA) was compared to HI for the detection of exposure to ferlavirus (Kania et al., 2000). Although there were titre differences between these two diagnostic tests, overall, there was agreement as to whether a sample was positive or negative.

## Immunohistochemistry (IHC)

Immunohistochemistry (IHC) requires the availability of animal-derived polyclonal or monoclonal antibodies that are specific to the virus under investigation (Sand *et al.*, 2004). The use of polyclonal antibodies makes this test difficult to standardise between laboratories (Homer *et al.*, 1995).

The detection of ferlavirus by IHC has been reported by various authors. Homer *et al.* (1995) inoculated rabbits with ferlavirus to produce a source of anti-ferlavirus polyclonal antibodies. These antibodies were then used to immunohistochemically identify ferlavirus antigen in formalin-fixed paraffin-embedded tissues. This study demonstrated that standard formalin-fixation practices did not prevent the identification of ferlavirus in infected tissues. However, fixation times were not always listed. Since this first report, IHC has been used to detect ferlavirus antigen in ferlavirus-infected Vero cells (Richter *et al.*, 1996), experimentally-inoculated Aruba Island rattlesnakes (*Crotalus unicolor*) (Jacobson *et al.*, 1997), a caiman lizard (*Draecena guianensis*) (Jacobson *et al.*, 2001), six snakes from the Canary Islands (Oros *et al.*, 2001) and three pit vipers (*Bothrops alternatus*) from Brazil (Kolesnikovas *et al.*, 2006).

## *In Situ* Hybridisation (ISH)

Two advantages that *in situ* hybridisation (ISH) has over IHC, is that

biologically-derived polyclonal or monoclonal antibodies are not needed and that viral transcripts can be detected (Sand *et al.*, 2004). Using oligonucleotides as probes, Sand *et al.* (2004) were able to identify segments of the ferlavirus haemagglutinin-neuraminidase attachment gene (HN) in the tissue sections of 11 out of 14 snakes that had histopathological findings that were consistent with a ferlavirus infection. The 14 samples were then tested by polymerase chain reaction (PCR) and the same 11 samples were positive. No further investigations of the three negative results were reported. In another report, West *et al.* (2001) used a generic avian paramyxovirus probe to detect ferlavirus in the brain of a Boelen's python *(Morelia boeleni*) with neurological signs.

## Polymerase Chain Reaction (PCR)

As mentioned in the previous section on *in situ* hybridisation (ISH), Sand *et al.* (2004) used PCR to identify ferlaviral RNA in FFPE tissues. Primer sequences that target the attachment gene (HN) were designed that produce relatively small amplicons: 153 nucleotides.

PCR is dependent on an adequate quantity and quality of viral RNA being present in the sample. In a hypothetical example where a cloacal swab was tested by PCR and the swab was collected from a snake infected with ferlavirus that is not shedding ferlavirus into its cloaca, the PCR result will be negative. The shedding pattern of ferlavirus is not

known (Jacobson and Origgi, 2007), so PCR results in live snakes that are negative may not always be accurate representations of the animal's disease status.

In 1999, primer sequences were designed and then successfully used for the detection of the polymerase (L) gene of ferlavirus (Ahne *et al.*, 1999b). This L gene primer set has been used for nucleic acid detection by several authors (Ahne et al., 1999b; Franke et al., 2001; Nogueira et al., 2002; Marschang et al., 2009; Papp et al., 2010a; Papp et al., 2010b; Abbas et al., 2011) and diagnostic laboratories (Table 7). In contrast to this, the haemagglutinin-neuraminidase (HN) gene has been targeted by other investigators (Sand *et al.*, 2004; Kolesnikovas *et al.*, 2006). Kurath *et al.* (2004) reported the order of conservation between paramyxoviral proteins (most conserved to least conserved): V-carboxy domain > L > M/F > N/HN > V > P. In agreement with this, Kindermann *et al.* (2001) found the L gene, from a selection of ferlaviruses, to be more conserved than the HN gene.

In one study, a PCR targeting the HN gene was performed on 47 clinical samples (swabs, organs) that had previously been determined to be positive for ferlavirus by an L gene PCR (Papp *et al.*, 2010a). Only 34% were positive when tested with the HN gene PCR. Because this study first screened samples with the L gene PCR, there was no opportunity that a sample could be discovered that was positive by the HN gene PCR

699 and negative by the L gene PCR. In another study, Kolesnikovas *et al.*
700 (2006) used an HN gene primer set (Ahne *et al.*, 1999b) to successfully
701 amplify ferlavirus from cell culture supernatant and infected tissues.
702 Some tissue samples were negative using this primer set but these
703 results were not pursued. Considering several reports have been able to
704 detect ferlaviruses using an L gene PCR but not with HN, F and/or U
705 gene PCRs (Ahne *et al.*, 1999b; Franke *et al.*, 2001; Marschang *et al.*,
706 2009; Papp *et al.*, 2010a), it seems reasonable to target the L gene in
707 preference to other genes.
708
709 Not all authors have used the primer sets designed by Ahne *et al.*
710 (1999b). In a retrospective study of 22 snakes from the Netherlands that
711 died with histological findings consistent with ferlavirus infection, ten
712 were positive for ferlavirus using newly designed primers (Kik *et al.*,
713 2004). Other studies have used novel degenerate primers to target the
714 fusion (F) (Franke et al., 2001; Franke et al., 2006) and "unknown" (U)
715 (Marschang *et al.*, 2009) genes of ferlavirus but neither primer set has
716 been used diagnostically.

## 717 Commercially Available Diagnostic Tests

718 The diagnostic tests for ferlavirus that are available to the clinical
719 practitioner are restricted to haemagglutination inhibition (HI),
720 polymerase chain reaction (PCR) and virus isolation (Table 7). To the
721 best of our knowledge, these tests are only offered on a commercial

basis in Europe, the United States of America, and Australia.

## Sunshine virus

In 2008, tissue and serum samples were collected following an outbreak of neurorespiratory disease in an Australian collection of 70 pythons. A syncytial-cell forming virus was isolated and using Illumina® high-throughput sequencing, the virus was identified as a novel paramyxovirus (Hyndman *et al.*, 2012a). The virus was named Sunshine virus after the geographical origin of the first isolate: the Sunshine Coast of Queensland, Australia. This virus represents the first paramyxovirus to be identified from a reptile that was not a ferlavirus. This virus has not been detected outside of Australia although testing thus far has been limited.

A set of PCR primers has since been designed that has been able to detect Sunshine virus in swabs and fresh and formalin-fixed paraffin-embedded tissues (Hyndman et al., 2012b). Sunshine virus has so far been detected in black-headed pythons (*Aspidites melanocephalus*), woma pythons (*A. ramsayi*), spotted pythons (*Antaresia maculosa*) and carpet pythons (*Morelia spilota* spp. and *M.bredli*). Clinical signs associated with Sunshine virus, like ferlavirus, are non-specific (e.g. lethargy, inappetance) and/or neurorespiratory in origin. Gross pathology is usually unremarkable. Histopathology reliably exhibits hindbrain white matter spongiosis and gliosis with extension to the

surrounding grey matter and neuronal necrosis is evident in severe cases. A mild bronchointerstitial pneumonia is seen in some snakes. In contrast to ferlavirus, which is most often detected in lung (Papp *et al.*, 2010a), Sunshine virus was detected most often in brain.

## Conclusion

Over the last 40 years, more than 50 papers have been published about the paramyxoviruses that infect reptiles. The majority of these are concerned with the ferlaviruses that infect snakes but recently, a diverse paramyxovirus that infects snakes, named Sunshine virus, has been described. Outbreaks of ferlavirus have been associated with significant morbidity and mortalities, and so it is important that herpetologists and veterinarians that work with reptiles are aware of the biology of these viruses and the clinical signs and pathological findings that are associated with infection. There are still important gaps in the knowledge concerning these viruses and their associated infections. For example, the incubation periods and shedding kinetics of the paramyxoviruses from reptilian hosts, and the survivability of the virus once outside the host, are all poorly understood and as consequences, choosing appropriate quarantine periods, proper sampling times and suitable sample types is problematic. It is our hope that this review will help future researchers of this area identify these knowledge gaps so they may contribute to this field as effectively as possible.

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1031

| Clinical Sign | Reference |
|---|---|
| **Nonspecific** | |
| Anorexia | (Jacobson *et al.*, 1992; Manvell *et al.*, 2000; Kolesnikovas *et al.*, 2006) |
| Regurgitation (occasional) | (Jacobson *et al.*, 1981; Jacobson *et al.*, 1992; Kolesnikovas *et al.*, 2006) |
| Mucoid diarrhoea or malodorous stools | (Jacobson *et al.*, 1981; Jacobson *et al.*, 1992; Kolesnikovas *et al.*, 2006) |
| Lethargy/Moribund | (Folsch and Leloup, 1976; Ahne *et al.*, 1987) |
| Sudden death | (Jacobson et al., 1981; Jacobson et al., 1997; Marschang et al., 2009; Papp et al., 2010) |
| **Respiratory** | |
| Not seen | (Jacobson *et al.*, 1992; West *et al.*, 2001) |
| Not described further | (Blahak, 1995; Kolesnikovas et al., 2006; Marschang et al., 2009) |
| Brown to haemorrhagic discharge from nostrils and/or trachea or in oral cavity | (Jacobson *et al.*, 1981; Jacobson *et al.*, 1997) |
| Stridor and/or respiratory noise | (Manvell *et al.*, 2000) |
| Pneumonia | (Blahak et al., 1991; Nogueira et al., 2002; Papp et al., 2010) |
| Clear mucus in mouth | (Potgieter *et al.*, 1987) |
| Clear nasal discharge | (Manvell *et al.*, 2000) |
| Mouth gaping | (Folsch and Leloup, 1976; Jacobson *et al.*, 1981) |
| **Neurological** | |
| Not described further | (Blahak, 1995; Papp et al., 2010) |
| Complete flaccid paralysis | (West *et al.*, 2001) |
| Decreased cutaneous sensation | (West *et al.*, 2001) |
| Head tremors | (Jacobson *et al.*, 1980; Jacobson *et al.*, 1992; Kolesnikovas *et al.*, 2006) |
| Abnormal posturing/disequilibrium i.e. opisthotonus (star gazing) or inability to right itself | (Folsch and Leloup, 1976; Jacobson et al., 1980; Blahak et al., 1991; Jacobson et al., 1992; Kolesnikovas et al., 2006; Papp et al., 2010) |

1032 Table 1 Clinical signs associated with ferlaviral infection. Only the references
1033 that report on original data are included here.
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1034

| Gross Pathology | Reference |
|---|---|
| None | (Jacobson *et al.*, 1997; Kolesnikovas *et al.*, 2006) |
| Pulmonary congestion or oedema | (Folsch and Leloup, 1976; Potgieter *et al.*, 1987; Oros *et al.*, 2001; Kolesnikovas *et al.*, 2006; Jacobson, 2007) |
| Haemorrhagic pneumonia | (Jacobson *et al.*, 1992; Jacobson *et al.*, 1997; Oros *et al.*, 2001; West *et al.*, 2001) |
| Blood in oral cavity or free in coelom | (Jacobson *et al.*, 1997; Jacobson, 2007) |
| White nodules on liver | (Jacobson *et al.*, 1992) |
| Mucoid or caseous exudate in the lung | (Jacobson et al., 1980; Jacobson et al., 1981; Blahak et al., 1991) |
| Diffuse to focal accumulations of caseous necrotic debris in pulmonary tissue | (Jacobson *et al.*, 1992; Oros *et al.*, 2001) |

1035 Table 2 Gross pathological changes seen associated with ferlaviral infection.
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1037

| Histopathological Change | Reference |
|---|---|
| **Respiratory** | |
| Moderate to diffuse amounts of cellular debris and exudate filling airways | (Jacobson *et al.*, 1980; Jacobson *et al.*, 1981) |
| Varying amounts of mixed inflammatory cells in the interstitium | (Jacobson *et al.*, 1980; Jacobson *et al.*, 1981; Potgieter *et al.*, 1987; Jacobson *et al.*, 1992; Jacobson *et al.*, 1997; Oros *et al.*, 2001; West *et al.*, 2001; Kolesnikovas *et al.*, 2006; Jacobson, 2007) |
| Gram negative microorganisms seen | (Homer *et al.*, 1995; Oros *et al.*, 2001; Jacobson, 2007) |
| Hyperplastic alveolar [faveolar] cells | (Potgieter et al., 1987; Homer et al., 1995; Jacobson et al., 1997; Oros et al., 2001; Jacobson, 2007) |
| Thickened pulmonary septae | (Homer *et al.*, 1995; Jacobson *et al.*, 1997; Jacobson, 2007) |
| Hyperplasia and often hypertrophied epithelium | (Jacobson *et al.*, 1981; Homer *et al.*, 1995) |
| Small numbers of pale eosinophilic intracytoplasmic (or not described) inclusions | (Jacobson et al., 1981; Potgieter et al., 1987; Blahak et al., 1991; Homer et al., 1995; Jacobson et al., 1997) |
| Giant cell formation | (Homer *et al.*, 1995; Kolesnikovas *et al.*, 2006) |
| Lesion severity decreases from cranial to middle to caudal lung area* | (Jacobson *et al.*, 1997) |
| **Neurological**[§] | |
| Eosinophilic intracytoplasmic inclusion bodies | (West *et al.*, 2001) |
| Demyelination and degeneration of axon fibers | (Jacobson *et al.*, 1980) |
| Multifocal neuronal degeneration | (West *et al.*, 2001) |
| Lymphohistiocytic neuritis of oesophagus | (West *et al.*, 2001) |
| Moderate axonal sheath ballooning | (Jacobson *et al.*, 1980) |
| Multifocal gliosis | (Jacobson *et al.*, 1980) |
| Perivascular cuffing in the brain | (Jacobson *et al.*, 1980; West *et al.*, 2001; Jacobson, 2007) |
| **Other** | |
| Intracytoplasmic inclusion bodies in the liver | (Blahak et al., 1991) |
| Pancreatitis and/or pancreatic necrosis and/or pancreatic fibrosis | (Jacobson *et al.*, 1980; Jacobson *et al.*, 1992; Kolesnikovas *et al.*, 2006; Jacobson, 2007) |

| | |
|---|---|
| Pancreatitic giant cell formation | (Kolesnikovas *et al.*, 2006) |
| Pyogranulomatous hepatitis | (Jacobson *et al.*, 1992) |
| Gram negative infections seen in many organs | (Jacobson *et al.*, 1992) |

Table 3 Histopathological changes seen associated with ferlaviral infection. *These findings were in snakes that were experimentally infected with ferlavirus by endotracheal inoculation. [§]Brain was not examined histologically in Jacobson *et al.* (1981), Potgieter *et al.* (1987), Jacobson *et al.* (1992), Homer *et al.* (1995) or Jacobson *et al.* (1997). It is unclear whether the brain was examined in Oros *et al.* (2001) and Kolesnikovas *et al.* (2006).

1046

| Quarantine Time | Reference |
|---|---|
| At least 30 days | (Jacobson *et al.*, 1980) |
| Minimum of 60 days | (Lloyd and Flanagan, 1991) |
| Minimum of 60-90 days | (Bronson and Cranfield, 2006) |
| Up to 90 days | (Gillespie, 2006) |
| 90 days | (Pasmans *et al.*, 2007) |
| Minimum of 90 days for animal raised in captivity | (Ritchie, 2006) |
| Minimally 90 days in a clinically healthy collection | (Jacobson *et al.*, 1992; Marschang and Chitty, 2004) |
| At least two months since the last death in an affected collection | (Jacobson *et al.*, 1992) |
| Minimum of three months with serology done at beginning and end | (Jacobson *et al.*, 1999) |
| Four months | (Hernandez-Divers, 2006) |
| Six months or 180 days | (Keeble, 2004; Ritchie, 2006; Rossi, 2006) |

1047 Table 4 The various quarantine periods that are recommended in the ferlaviral

1048 literature.

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| Virus Isolation Techniques | Reference |
|---|---|
| **Successful attempts** | |
| Sputum inoculated directly onto Vero cells | (Nogueira *et al.*, 2002) |
| Fulfilling Koch's postulates: Vero cell-adapted ferlavirus was transmitted to naïve snakes and then lung homogenates were recovered at necropsy and used to reisolate ferlavirus onto Vero cells | (Jacobson *et al.*, 1997) |
| Lung suspension inoculated onto fathead minnow skin cells (FHM, a piscine cell line), hamster kidney cells (BHK-21), swine testicular cells (ST), Vero cells and primary bovine turbinate cells (BTU) | (Potgieter *et al.*, 1987) |
| Lung suspension inoculated into embryonated snake eggs and then subcultured onto nine reptilian and four mammalian cell lines | (Clark *et al.*, 1979) |
| Pooled and/or individual snake organs inoculated onto monolayer of VH2 and/or IgH2 cells | (Jacobson *et al.*, 1980; Jacobson *et al.*, 1981; Ahne *et al.*, 1987; Blahak, 1994; Homer *et al.*, 1995; Kolesnikovas *et al.*, 2006; Papp *et al.*, 2010a; Abbas *et al.*, 2011) |
| Various lizard organs inoculated onto VH2 and TH1 cells. After eight passages in TH1 cells, was adapted to Vero cells | (Jacobson *et al.*, 2001) |
| Ferlavirus replicating in VH2 cells adapted to Vero cells | (Blahak, 1995; Richter et al., 1996; Mayr et al., 2000) |
| Ferlavirus replicating in IgH2 cells adapted to Vero cells | (Richter *et al.*, 1996; Mayr *et al.*, 2000) |
| Ferlavirus replicating in IgH2 cells adapted to chicken embryo fibroblasts (LSCC-H32) and embryonated chicken eggs | (Ahne et al., 1999) |
| Ferlavirus replicating in VH2 cells adapted to chicken embryo fibroblasts | (Blahak, 1994) |
| Ferlavirus replicating in VH2 cells adapted to Madin Darby bovine kidney cells (MDBK) and rabbit kidney cells (RK-13) | (Blahak, 1995) |
| Lung suspension inoculated into snake embryo fibroblasts | (Manvell *et al.*, 2000) |
| **Unsuccessful attempts*** | |
| Tortoise organ suspensions inoculated onto TH1 and VH2 cells | (Papp *et al.*, 2010b) |

| | |
|---|---|
| Pooled and/or individual snake organs inoculated onto monolayer of IgH2 cells | (Jacobson *et al.*, 1980) |
| Lung suspension inoculated into embryonated snake eggs and then subcultured onto three piscine cell lines | (Clark *et al.*, 1979) |
| Lung suspension inoculated into the allantois of SPF embryonated chicken eggs and onto chicken embryo fibroblasts and Vero cells | (Manvell *et al.*, 2000) |
| Lung suspension inoculated onto VH2 cells and feline kidney cells (CRFK) | (Potgieter *et al.*, 1987) |
| Reptilian paramyxovirus replicating in fathead minnow cells could not be subcultured into the allantois of SPF embryonated chicken eggs | (Potgieter *et al.*, 1987) |

Table 5 The methods that have been reported in the literature to isolate paramyxoviruses from reptiles. VH2 = viper heart cells. TH1 = terrapene heart cells. IgH2 = iguana heart cells. SPF = specific pathogen free. *Only those attempts where a paramyxovirus was successfully isolated using a different biological substrate are mentioned. This is to exclude the possibility that the unsuccessful attempt was because there was simply no virus in the inoculum. However, the possibility that the virus *did* grow in these cells but was not detected cannot be ruled out as further testing (e.g. polymerase chain reaction) was not performed.

| Cytopathic Effect | Cell line | | | | | | |
|---|---|---|---|---|---|---|---|
| | Viper heart cells (VH2) | Terrapene heart cells (TH1) | Vero cells | Iguana heart cells (IgH2) | Chicken embryo fibroblasts (LSCC-H32) | Fathead minnow skin (FHM) | Various[§] |
| Syncytial/giant cell formation | (Abbas *et al.*, 2011)[ϕ] (Jacobson *et al.*, 1980; Jacobson *et al.*, 1981; Blahak, 1995; Homer *et al.*, 1995; Jacobson *et al.*, 2001) | (Jacobson *et al.*, 2001)[#] | (Richter *et al.*, 1996)[1] (Mayr *et al.*, 2000)[2] (Jacobson *et al.*, 2001)[3] | (Ahne *et al.*, 1987; Ahne *et al.*, 1999b) | (Ahne *et al.*, 1999a)[2] | (Potgieter *et al.*, 1987)* | (Clark *et al.*, 1979) |
| Cytoplasmic inclusion bodies | (Jacobson *et al.*, 1981) | | (Mayr *et al.*, 2000)[2] | | | | |
| Cell lysis/monolayer destruction | (Abbas *et al.*, 2011)[ϕ] (Jacobson *et al.*, 1981; Blahak, 1995; Homer *et al.*, 1995; Kolesnikovas *et al.*, 2006) | | (Mayr *et al.*, 2000)[2] | (Ahne *et al.*, 1987; Ahne *et al.*, 1999b) | (Ahne *et al.*, 1999a)[2] | (Potgieter *et al.*, 1987)* | (Clark *et al.*, 1979) |
| Elongation of cell processes | (Homer *et al.*, 1995) | | | | | | |
| Cell vacuolisation | | | | | (Ahne *et al.*, 1999a)[2] | | |
| Cell rounding | (Kolesnikovas *et al.*, 2006) | | | | | | |

Table 6 The cytopathic effects of paramyxoviruses isolated from snakes and a lizard that have been described in the literature. [§] This isolate displayed CPE in nine reptilian and four mammalian cell lines after first being passaged through an embryonated snake egg. [1] Adapted from VH2 cells. [2] Adapted from IgH2 cells. [3] Adapted from TH1 cells. * Undescribed cytopathic changes were also seen when this isolate was propagated in hamster kidney cells (BHK-21), swine testicular cells (ST), Vero cells and primary bovine turbinate cells (BTU). [#] This isolate was obtained from a caiman lizard (*Draecena guianensis*). [ϕ] Tissue homogenates were inoculated onto VH2 and IgH2 cells but cell line that the isolate was successful isolated with is not specified.

1071

| Laboratory | Reference |
|---|---|
| **Haemagglutination Inhibition (HI)** | |
| Hohenheim University, Germany | (Heard *et al.*, 2004) |
| Chemical and Veterinary Investigation Office (CVUA) of East Westphalia-Lippe, Germany | (R. Marschang, pers. comm.) |
| Veterinary Laboratories Agency (VLA), UK | (Keeble, 2004) |
| The University of Florida, USA | (Heard *et al.*, 2004) |
| The University of Tennessee, USA | (Heard *et al.*, 2004) |
| Texas State Diagnostic Laboratory, USA | (Ritchie, 2006) |
| **Polymerase Chain Reaction (PCR)** | |
| Hohenheim University, Germany* | (R. Marschang, pers. comm.) |
| Chemical and Veterinary Investigation Office (CVUA) of East Westphalia-Lippe, Germany* | (R. Marschang, pers. comm.) |
| IDEXX Vet Med Labor, Germany | http://www.idexx.de |
| Laboklin, Germany | http://www.laboklin.de/ |
| The University of Florida, USA* | (Heard *et al.*, 2004) |
| Murdoch University, Australia* | (T. Hyndman, pers. comm.) |
| **Virus Isolation** | |
| Hohenheim University, Germany | (R. Marschang, pers. comm.) |
| Chemical and Veterinary Investigation Office (CVUA) of East Westphalia-Lippe, Germany | (R. Marschang, pers. comm.) |

1072 Table 7 Diagnostic tests for ferlavirus that are commercially available. *Known
1073 to use the primer pairs designed by Ahne *et al.* (1999b) targeting the L gene.
1074