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# 1 **Paramyxoviruses in Reptiles: A Review**

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## 13 **Abstract**

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

## 27 **Keywords**

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

## 29 **Introduction**

30 A wide range of viruses have been detected in reptiles throughout the  
31 world and the interested reader is referred to the reviews by Wellehan  
32 and Johnson (2005), Jacobson (2007), Marschang (2011) and Ariel (2011)  
33 for more general overviews of these viruses. This review will focus on  
34 the paramyxovirus infections of reptiles. Since an outbreak of  
35 neurorespiratory disease in a Swiss serpentarium that was associated  
36 with a paramyxovirus (Folsch and Leloup, 1976), reptilian  
37 paramyxoviruses have been described in other regions of Europe (Ahne  
38 et al., 1987; Blahak, 1995; Manvell et al., 2000; Franke et al., 2001), USA  
39 (Jacobson et al., 1980; Jacobson et al., 1981; Potgieter et al., 1987;  
40 Richter et al., 1996) and Brazil (Nogueira et al., 2002; Kolesnikovas et al.,  
41 2006). Most recently, a novel paramyxovirus, named Sunshine virus,  
42 was described in Australian pythons (Hyndman et al., 2012a; Hyndman  
43 et al., 2012b) and so it is important to review the established and  
44 emerging areas in this field.

## 45 **Taxonomy of Reptilian Paramyxoviruses**

46 Members of the family Paramyxoviridae are currently divided into two  
47 subfamilies: Pneumovirinae and Paramyxovirinae (ICTV, 2013).

48 Paramyxovirinae currently contains seven genera, one of which is the

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

## 55 **Ferlavirus**

56 The literature has not shown conformity in how it refers to ferlaviruses.  
57 Since the first characterisation of a snake paramyxovirus that was  
58 named Fer de Lance Virus (FDLV) (Clark et al., 1979), the term ophidian  
59 paramyxovirus (often abbreviated to OPMV or oPMV) has also been  
60 used to describe the paramyxoviruses found in snakes (Lloyd and  
61 Flanagan, 1991; Homer et al., 1995; Jacobson et al., 1997; Manvell et al.,  
62 2000; Kindermann et al., 2001; Oros et al., 2001; Nogueira et al., 2002;  
63 Kolesnikovas et al., 2006). In 2009, a proposal was put forward by  
64 Kurath to the International Committee on Taxonomy of Viruses (ICTV) to  
65 create the new genus Ferlavirus with Fer-de-Lance paramyxovirus (the  
66 same virus as FDLV) as its type species. This proposal has been accepted  
67 by the ICTV (2013) and all future work should refer to these viruses as  
68 ferlaviruses. By avoiding the general term “ophidian paramyxovirus”,  
69 Sunshine virus and the ferlaviruses of snakes can be unambiguously  
70 delineated.

71

72 Folsch and Leloup (1976) produced the first report of a reptilian  
73 paramyxovirus following an outbreak of neurorespiratory disease in a  
74 Swiss serpentarium. The physicochemical traits of this first isolate were  
75 then characterised and described by Clark et al. (1979). The origin of the  
76 ferlaviruses is unknown but one reference provides further insight. A  
77 personal communication mentioned in a paper by Kolesnikovas et al.  
78 (2006) states that the Brazilian lancehead vipers (*Bothrops moojeni* but  
79 incorrectly referred to as Fer-de-Lance vipers [*B. atrox*] in earlier works)  
80 in the Swiss serpentarium originated from Brazil. No further information  
81 is provided.

82

83 The entire genome of Fer-de-Lance paramyxovirus has been sequenced  
84 (Kurath et al., 2004). The genome is 15,378 nucleotides long and is  
85 made up of seven distinct genes: 3' – Nucleocapsid (N) – Unknown (U) –  
86 Phosphoprotein/Protein V (P/V) – Matrix (M) – Fusion (F) –  
87 Haemagglutinin-Neuraminidase (HN) – RNA-Dependent RNA  
88 Polymerase (L). The fusion gene has been analysed by others (Franke et  
89 al., 2006). Several authors have analysed the phylogenetic relationships  
90 that exist between ferlaviruses (Ahne et al., 1999b; Franke et al., 2001;  
91 Kindermann et al., 2001; Marschang et al., 2009; Papp et al., 2010a;  
92 Papp et al., 2010b; Abbas et al., 2011) while others have compared the  
93 ferlaviruses to other paramyxoviruses (Junqueira de Azevedo et al.,  
94 2001; Kurath et al., 2004; Marschang et al., 2009). These studies  
95 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

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

125  
126 The incongruence that exists in the conclusions of the studies on  
127 ferlaviral serological relatedness could be explained by a serological  
128 unrelatedness between the various ferlaviral isolates used in these  
129 studies. Serological unrelatedness between ferlaviral isolates has been  
130 shown in at least two studies (Marschang et al., 2002; Allender et al.,  
131 2008). In general, however, it seems reasonable to conclude that the  
132 serological relatedness of the ferlaviruses to other paramyxoviruses is  
133 limited at most.

134  
135 All ferlaviruses are believed to have neuraminidase activity. Using a  
136 substrate that is specifically cleaved by neuraminidase into an intensely  
137 fluorescent product (Yolken et al., 1980), significant neuraminidase  
138 activity was detected in three isolates of ferlavirus (Richter et al., 1996).  
139 Clark et al. (1979) also demonstrated the presence of neuraminidase  
140 activity in ferlavirus. After haemagglutinating chicken and guinea pig  
141 erythrocytes with ferlavirus it was observed that these erythrocytes  
142 could not be re-agglutinated by ferlavirus, implying that the virus has a  
143 receptor destroying enzyme (neuraminidase).



## 144 ***Clinical Findings Associated with Infection***

145 Ferlaviral infections have been associated with highly pathogenic  
146 disease outbreaks (Folsch and Leloup, 1976; Jacobson et al., 1980;  
147 Jacobson et al., 1981; Jacobson et al., 1992; Kolesnikovas et al., 2006).  
148 Infection has been detected in several snake families: Colubridae,  
149 Elapidae, Viperidae, Crotalidae, Boidae and Pythonidae (Jacobson et al.,  
150 1997; Ahne et al., 1999b; Oros et al., 2001). One report described the  
151 clinical signs associated with ferlaviral infection as being variable, often  
152 non-specific, and occasionally subtle (Sand et al., 2004). When clinical  
153 signs can be attributed to a particular organ system, they are most  
154 commonly localised to the respiratory tract (Jacobson, 2007) but there  
155 are also reports about snakes suffering from neurological disease. Table  
156 1 outlines the clinical signs reported by various authors.

157  
158 In 1991, Lloyd and Flanagan described the clinical manifestations of  
159 ferlaviral infection as fitting into three discrete clinical syndromes:  
160 snakes affected acutely or peracutely; “poor doers”; and, clinically-  
161 healthy animals that shed virus in the face of high antibody titres. These  
162 observations were based on clinical experience and were not from a  
163 controlled study.

## 164 ***Gross Pathology***

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

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

### 169 ***Histopathology***

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

184  
185 Through the use of immunohistochemistry, Homer et al. (1995) was  
186 able to localise pulmonary infections to the luminal surface and  
187 cytoplasm of faveolar epithelium. With a similar purpose, Sand et al.  
188 (2004) used in situ hybridisation to locate ferlavirus in a variety of  
189 infected organs. Virus was intranuclear in the brain while being

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

### 193 ***Non-Ferlaviral Microbiological Findings***

194 A number of authors have reported on the non-ferlaviral microbiological  
195 findings in snakes infected with ferlavirus. Bacterial cultures from  
196 various organs have identified several Gram negative bacterial  
197 pathogens: Aeromonas, Citrobacter, Escherichia, Enterobacter,  
198 Morganella, Proteus, Providencia, Pseudomonas, Salmonella and  
199 Serratia (Folsch and Leloup, 1976; Jacobson et al., 1981; Blahak et al.,  
200 1991; Jacobson et al., 1992; Homer et al., 1995; Oros et al., 2001;  
201 Kolesnikovas et al., 2006; Jacobson, 2007). In one of these studies,  
202 fungal elements could not be cultured (Kolesnikovas et al., 2006). It has  
203 been suggested that ferlaviral infections may be immunosuppressive,  
204 possibly due to lymphoid depletion (Oros et al., 2001), allowing  
205 secondary bacterial invaders (Kolesnikovas et al., 2006). So while it is  
206 recommended that concurrent complicating bacterial infections be  
207 treated as early as possible (Jacobson et al., 1992; Kolesnikovas et al.,  
208 2006), antibiotic use may not provide any improvement in already  
209 affected snakes (Folsch and Leloup, 1976; Jacobson et al., 1981).

210

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

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

### 219 ***Transmission***

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

227  
228 Pasmans et al. (2008) states that ferlavivirus is easily transmitted through  
229 both aerosols and contact, and terraria for individually housed snakes  
230 provide little defense against the transmission of ferlavivirus. It has also  
231 been suggested that ferlavivirus may be transmitted from snake to snake  
232 by direct contact, respiratory secretions, fomites and ectoparasites  
233 (especially mites) (Hernandez-Divers, 2006). In the first outbreak  
234 described by Folsch and Leloup (1976), the infection spread from the  
235 enclosures closest to the doors in two different rooms. It was

236 hypothesised that spread had occurred either by aerosol or via the  
237 keepers. Considering that ferlaviruses have been isolated from the  
238 sputum of a rattlesnake (*Crotalus durissus terrificus*) (Nogueira et al.,  
239 2002), oral and cloacal swabs from corn snakes (*Pantherophis guttatus*)  
240 (Abbas et al., 2011) and detected by polymerase chain reaction (PCR) in  
241 oral and cloacal swabs (Papp et al., 2010a), it is reasonable to assume  
242 that ferlavirus can be transmitted between snakes by both oral  
243 secretions and cloacal excretions. To the best of our knowledge,  
244 ferlavirus has not been isolated, or detected by PCR, from fomites or  
245 ectoparasites. There are currently no reports concerning vertical  
246 transmission of ferlavirus (Pasmans et al., 2008).

247

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

253

254 The shedding patterns of ferlavirus are unknown (Jacobson and Origi,  
255 2007). Although Lloyd and Flanagan (1991) state that some snakes are  
256 capable of shedding virus for an extended period of time, this claim is  
257 based on the observation that some snakes have significant  
258 haemagglutination inhibition (HI) antibody titres for five months or  
259 more while simultaneously “causing” seroconversion in cagemates

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

## 264 ***Treatment***

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

275  
276 Symptomatic treatment has generally been provided by broad spectrum  
277 antibiotics (Kolesnikovas et al., 2006). Bronson and Cranfield (2006)  
278 have stated that the survival time of snakes infected with ferlavirus is  
279 improved by targeting secondary protozoal and bacterial infections.

280 **Prevention**

281 **Quarantine**

282 To prevent the introduction of ferlavirus into a collection it is  
283 recommended that new animals are only introduced after a period of  
284 quarantine. Recommended lengths of quarantine vary between  
285 references (Table 4) but there is a general trend where modern  
286 recommendations are for longer periods of time. All of the  
287 recommendations are empirical due to the limited information that is  
288 available about ferlaviral transmission and environmental viability.  
289  
290 Various authors have provided specific recommendations about caring  
291 for a collection consisting of resident and quarantined animals  
292 (Jacobson et al., 1999; Marschang and Chitty, 2004; Ritchie, 2006;  
293 Pasmans et al., 2008). During quarantine, agent-specific testing can be  
294 used to help determine if a snake has been exposed to, or is infected  
295 with, ferlavirus.

296 **Vaccination**

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

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

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

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

314

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



326 was not performed.

## 327 ***Ferlavirus in Non-Snake Reptilian Hosts***

### 328 **Lizards**

329 Most of the paramyxoviruses that have been described in lizards have  
330 not been associated with overt disease. A paramyxovirus was isolated  
331 from a false tegu (*Callopistes maculatus*) in 1988 (Ahne and Neubert,  
332 1991) that was later identified as a ferlavirus by sequence analysis  
333 (Ahne et al., 1999b). Similarly, a paramyxovirus was isolated from the  
334 mouth of an apparently healthy monitor lizard (*Varanus prasinus*) that  
335 was part of a reptile collection that had suffered an outbreak of  
336 ferlavirus in its snake population (Gravendyck et al., 1998).

337

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

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

357

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

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

376

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

### 393 **Chelonians**

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

396 from three species: *Testudo graeca* (Greek tortoise), *T. hermanni*  
397 (Herman's tortoise) and *Geochelone elegans* (Indian star tortoise).  
398 Seven of these 34 tortoises were showing signs of rhinitis at the time of  
399 blood sampling. There was little correlation between anti-Sendai virus  
400 HI titre and the presence of clinical signs. The authors did not attempt  
401 to isolate a paramyxovirus from any of these tortoises. In another study,  
402 a collection of Mediterranean tortoises (*Testudo graeca* and *T. hermanni*)  
403 were imported from Turkey to Switzerland and upon arrival many were  
404 found to have a viral dermatitis (Zangger et al., 1991). Light microscopy  
405 revealed intracytoplasmic inclusions in the stratum germinativum and  
406 under electron microscopy, paramyxovirus-like particles were identified.  
407  
408 In 1990, Oettle et al. (1990) reported on the death of 31 out of 83  
409 African tortoises from four species: *Psammobates tentorius* (tent  
410 tortoise), *Homopus areolatus* (beaked cape tortoise), *Chersina angulata*  
411 (bowsprit tortoise) and *Geochelone pardalis* (leopard tortoise). Affected  
412 tortoises displayed a wide range of clinical signs and pathological  
413 changes but anorexia, progressive lethargy, severe ascites, hepatitis,  
414 pneumonia and necrotic pseudomembranous stomatitis were seen  
415 most commonly. Paramyxovirus-like inclusions were identified under  
416 light microscopy in oesophageal cells from one tortoise. It is likely that a  
417 range of disease processes featured in this outbreak and whether a  
418 paramyxovirus was present, and if so, the role it played, is unclear.  
419

420 In 1993, Witte found three out of 128 tortoises from ten different  
421 collections revealed antibody titres against a snake paramyxovirus.  
422 Titres were 1:16 in two Russian tortoises (*Agrionemys* [Testudo]  
423 *horsfieldii*) and 1:32 in a Greek tortoise (*Testudo graeca*).  
424  
425 In 1999, a ferlavirus was isolated from a Hermann's tortoise (*Testudo*  
426 *hermanni*) suffering from pneumonia (Marschang et al., 2009). The  
427 identity of this ferlavirus was confirmed by sequence analysis. More  
428 recently, several different ferlaviruses were detected by PCR from  
429 various organs of a leopard tortoise (*Geochelone pardalis babcocki*)  
430 suffering from respiratory distress (Papp et al., 2010b). There were large  
431 amounts of mucopurulent discharge from its nares and mouth and on  
432 necropsy, the lungs were bilaterally consolidated and filled with thick  
433 serous exudate. The ferlaviruses detected by PCR could not be isolated  
434 into cell culture.  
435  
436 In the most recent study on ferlaviruses in tortoises, tortoise plasma  
437 was screened for antibodies against ferlaviruses using  
438 haemagglutination inhibition testing. Antibodies were found in several  
439 tortoise species from several European countries (Rösler et al., 2013).

#### 440 **Crocodiles**

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

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

447

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

#### 455 ***Zoonotic Potential***

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

466 2000). Clark et al. (1979) comprehensively investigated the susceptibility  
467 of mice to infection with ferlavirus and could not detect any clinical or  
468 histological evidence of disease. Based on this information it would  
469 seem unlikely that ferlavirus would pose a serious zoonotic risk to  
470 human health.

### 471 ***Diagnostic Tests***

### 472 **Virus Isolation**

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

### 486 **Electron Microscopy**

487 Many authors have utilised transmission electron microscopy (TEM) to

488 identify reptilian paramyxoviruses (Lunger and Clark, 1978; Clark et al.,  
489 1979; Jacobson et al., 1980; Jacobson et al., 1981; Ahne et al., 1987;  
490 Potgieter et al., 1987; Richter et al., 1996; Jacobson et al., 1997; Manvell  
491 et al., 2000; Franke et al., 2001; Jacobson et al., 2001; West et al., 2001).  
492 Ferlaviruses are medium-sized, have spiked envelopes and can be  
493 spherical to pleomorphic in morphology (Jacobson and Samuelson,  
494 2007). The nucleocapsid of a paramyxovirus forms the core of the virion  
495 and has a distinct “herring bone” appearance (Ahne and Mayr, 2000).  
496 Spherical and filamentous forms of ferlavirus have been seen budding  
497 from infected cells (Jacobson et al., 1997).

498  
499 Inclusion bodies have been identified under light microscopy in snake  
500 tissue infected with ferlavirus (Jacobson et al., 1981; Potgieter et al.,  
501 1987; Homer et al., 1995; Jacobson et al., 1997; West et al., 2001).  
502 Ultrastructural assessment has shown these inclusions to be comprised  
503 of nucleocapsid strands (Jacobson et al., 1981; Jacobson et al., 1997).

#### 504 **Haemagglutination (HA) Assays**

505 Large quantities of virus are needed for macroscopic haemagglutination  
506 so this method is considered to be relatively insensitive (Quinn et al.,  
507 2002). In one study, ferlavirus-infected tissue homogenates did not  
508 haemagglutinate chicken erythrocytes but ferlavirus could be isolated  
509 onto viper heart cells and be detected by polymerase chain reaction  
510 (Kolesnikovas et al., 2006). Only after replication in viper heart cells,



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

514

### 515 **Haemagglutination Inhibition (HI)**

516 Haemagglutination inhibition (HI) has been used widely as a serological  
517 test for the detection of exposure to ferlavirus (Jacobson et al., 1981;  
518 Potgieter et al., 1987; Jacobson et al., 1991; Jacobson et al., 1992;  
519 Brousset et al., 1994; Blahak, 1995; Richter et al., 1996; Jacobson et al.,  
520 1997; Gravendyck et al., 1998; Manvell et al., 2000; Calle et al., 2001;  
521 Jacobson et al., 2001; Marschang et al., 2002; Lloyd et al., 2005;  
522 Allender et al., 2006; Allender et al., 2008) and is offered commercially  
523 by several diagnostic laboratories (Table 7).

524

525 Various vertebrate erythrocytes have been compared to each other in  
526 their ability to haemagglutinate three isolates of ferlavirus (Richter et al.,  
527 1996). Chicken and guinea pig erythrocytes reliably haemagglutinated  
528 these three isolates and outperformed sheep, human type-O and rabbit  
529 erythrocytes. Many, if not all, of the laboratories that offer HI  
530 commercially utilise chicken or guinea pig erythrocytes as markers of  
531 ferlavirus-induced haemagglutination (Allender et al., 2008).

532

533 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

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

563

564 In an outbreak of ferlavirus in a zoological collection, Jacobson et al.  
565 (1992) tested 31 snakes for the presence of anti-ferlavirus antibodies by  
566 HI. Twelve snakes showed positive titres (greater than 10) and these  
567 animals were then retested a number of times over the next year. Many  
568 cases showed high titres (5,120 to greater than 20,480) that decreased  
569 to low titres (below 100) over three to seven months.

570

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

577

578 An HI titre is complicated by a long list of variables: the antibody's  
579 affinity to the antigen, the integrity of the antigen being used, the  
580 availability of antibody in the serum, the preservation of the sample and  
581 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)

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

#### 614 **Non-haemagglutination Inhibition Antibody Assays**

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

629 **Immunohistochemistry (IHC)**

630 Immunohistochemistry (IHC) requires the availability of animal-derived  
631 polyclonal or monoclonal antibodies that are specific to the virus under  
632 investigation (Sand et al., 2004). The use of polyclonal antibodies makes  
633 this test difficult to standardise between laboratories (Homer et al.,  
634 1995).

635

636 The detection of ferlavirus by IHC has been reported by various authors.  
637 Homer et al. (1995) inoculated rabbits with ferlavirus to produce a  
638 source of anti-ferlavirus polyclonal antibodies. These antibodies were  
639 then used to immunohistochemically identify ferlavirus antigen in  
640 formalin-fixed paraffin-embedded tissues. This study demonstrated that  
641 standard formalin-fixation practices did not prevent the identification of  
642 ferlavirus in infected tissues. However, fixation times were not always  
643 listed. Since this first report, IHC has been used to detect ferlavirus  
644 antigen in ferlavirus-infected Vero cells (Richter et al., 1996),  
645 experimentally-inoculated Aruba Island rattlesnakes (*Crotalus unicolor*)  
646 (Jacobson et al., 1997), a caiman lizard (*Draecena guianensis*) (Jacobson  
647 et al., 2001), six snakes from the Canary Islands (Oros et al., 2001) and  
648 three pit vipers (*Bothrops alternatus*) from Brazil (Kolesnikovas et al.,  
649 2006).

650 ***In Situ* Hybridisation (ISH)**

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

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

#### 664 **Polymerase Chain Reaction (PCR)**

665 As mentioned in the previous section on in situ hybridisation (ISH), Sand  
666 et al. (2004) used PCR to identify ferlaviral RNA in FFPE tissues. Primer  
667 sequences that target the attachment gene (HN) were designed that  
668 produce relatively small amplicons: 153 nucleotides.  
669  
670 PCR is dependent on an adequate quantity and quality of viral RNA  
671 being present in the sample. In a hypothetical example where a cloacal  
672 swab was tested by PCR and the swab was collected from a snake  
673 infected with ferlavirus that is not shedding ferlavirus into its cloaca, the  
674 PCR result will be negative. The shedding pattern of ferlavirus is not

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

678

679 In 1999, primer sequences were designed and then successfully used for  
680 the detection of the polymerase (L) gene of ferlavirus (Ahne et al.,  
681 1999b). This L gene primer set has been used for nucleic acid detection  
682 by several authors (Ahne et al., 1999b; Franke et al., 2001; Nogueira et  
683 al., 2002; Marschang et al., 2009; Papp et al., 2010a; Papp et al., 2010b;  
684 Abbas et al., 2011) and diagnostic laboratories (Table 7). In contrast to  
685 this, the haemagglutinin-neuraminidase (HN) gene has been targeted by  
686 other investigators (Sand et al., 2004; Kolesnikovas et al., 2006). Kurath  
687 et al. (2004) reported the order of conservation between paramyxoviral  
688 proteins (most conserved to least conserved): V-carboxy domain > L >  
689 M/F > N/HN > V > P. In agreement with this, Kindermann et al. (2001)  
690 found the L gene, from a selection of ferlaviruses, to be more conserved  
691 than the HN gene.

692

693 In one study, a PCR targeting the HN gene was performed on 47 clinical  
694 samples (swabs, organs) that had previously been determined to be  
695 positive for ferlavirus by an L gene PCR (Papp et al., 2010a). Only 34%  
696 were positive when tested with the HN gene PCR. Because this study  
697 first screened samples with the L gene PCR, there was no opportunity  
698 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

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

## 723 **Sunshine virus**

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

734

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

745 surrounding grey matter and neuronal necrosis is evident in severe  
746 cases. A mild bronchointerstitial pneumonia is seen in some snakes. In  
747 contrast to ferlavirus, which is most often detected in lung (Papp et al.,  
748 2010a), Sunshine virus was detected most often in brain.

## 749 **Conclusion**

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

768 **References**

- 769 Abbas, M.D., Marschang, R.E., Schmidt, V., Kasper, A., Papp, T., 2011, A  
770 unique novel reptilian paramyxovirus, four adenovirus types and a  
771 reovirus identified in a concurrent infection of a corn snake  
772 (*Pantherophis guttatus*) collection in Germany. *Veterinary Microbiology*  
773 150, 70-79.
- 774 Ahne, W., Adrian, J., Mayr, A., 1999a, Replication of Reptilian  
775 Paramyxovirus in Avian Host Systems. *Journal of Veterinary Medicine B*  
776 46, 57-62.
- 777 Ahne, W., Batts, W.N., Kurath, G., Winton, J.R., 1999b, Comparative  
778 Sequence Analyses of Sixteen Reptilian Paramyxoviruses. *Virus Research*  
779 63, 65-74.
- 780 Ahne, W., Mayr, A., 2000, Reptilian Paramyxoviruses Induce Cytokine  
781 Production in Human Leukocytes. *Comparative Immunology,*  
782 *Microbiology & Infectious Diseases* 23, 9-13.
- 783 Ahne, W., Neubert, W., 1991. Isolation of paramyxovirus-like agents  
784 from teju (*Callisotes maculatus*) and python (*Python regius*). In:  
785 *Proceedings of the 2nd International Symposium of Viruses in Lower*  
786 *Vertebrates, Oregon State University, OR, pp. 203-210.*
- 787 Ahne, W., Neubert, W.J., Thomson, I., 1987, Reptilian Viruses: Isolation  
788 of Myxovirus-like Particles from the Snake *Elaphe oxycephala*. *Zentralbl*  
789 *Veterinarmed B* 34, 607-612.
- 790 Allender, M.C., Mitchell, M.A., Dreslik, M.J., Phillips, C.A., Beasley, V.R.,  
791 2008, Measuring agreement and discord among hemagglutination  
792 inhibition assays against different ophidian paramyxovirus strains in the  
793 Eastern massasauga (*Sistrurus catenatus catenatus*). *Journal of Zoo and*  
794 *Wildlife Medicine* 39, 358-361.
- 795 Allender, M.C., Mitchell, M.A., Phillips, C.A., Grusznski, K., Beasley, V.R.,  
796 2006, Hematology, Plasma Biochemistry, and Antibodies to Select  
797 Viruses in Wild-Caught Eastern Massasauga Rattlesnakes (*Sistrurus*  
798 *catenatus catenatus*) from Illinois. *Journal of Wildlife Diseases* 42, 107-  
799 114.
- 800 Alymova, I.V., Taylor, G., Portner, A., 2005, Neuraminidase Inhibitors as  
801 Antiviral Agents. *Current Drug Targets - Infectious Diseases* 5, 401-409.
- 802 Ariel, E., 2011, Viruses in reptiles. *Veterinary Research* 42, 100.
- 803 Blahak, S., 1994. Untersuchungen zum Vorkommen von Paramyxoviren  
804 bei Schlangen und Charakterisierung ausgewählter Isolate. Justus-

- 805 Liebig-Universität Giessen,
- 806 Blahak, S., 1995, Isolation and Characterisation of Paramyxoviruses from  
807 Snakes and their Relationship to Avian Paramyxoviruses. Zentralbl  
808 Veterinarmed B. 42, 216-224.
- 809 Blahak, S., Göbel, T., Alexander, D.J., Manvell, R., 1991. Some  
810 Investigations of the Occurrence of Paramyxoviruses in Snakes in  
811 German Zoos. In: Proceedings of the 4th International Colloquium on  
812 Pathology and Medicine of Reptiles and Amphibians, Bad Nauheim,  
813 Germany, pp. 17-24.
- 814 Boyer, T.H., Garner, M.M., Latimer, K.S., 2005, Paramyxovirus Infection  
815 in a Thai Water Dragon (*Physignathus concinns*). Proceedings of the  
816 12th Annual Meeting of the Association of Reptilian and Amphibian  
817 Veterinarians, 93-95.
- 818 Bronson, E., Cranfield, M.R., 2006, Paramyxovirus, In: Reptile Medicine  
819 and Surgery. Elsevier, St. Louis, pp. 858-861.
- 820 Brousset, D., Lewy, C., Yarto, E., 1994. Detection of Antibodies Against  
821 Paramyxovirus (OPMV) on Mexican Captive Snakes. In: Proceedings of  
822 the Association of Reptilian and Amphibian Veterinarians, p. 101.
- 823 Calle, P.P., Rivas, J., Munoz, M., Thorbjarnarson, J., Holmstrom, W.,  
824 Karesh, W.B., 2001, Infectious Disease Serologic Survey in Free-Ranging  
825 Venezuelan Anacondas (*Eunectes murinus*). Journal of Zoo and Wildlife  
826 Medicine 32, 320-323.
- 827 Chakrabarti, S., Collingham, K.E., Holder, K., Fegan, C.D., Osman, H.,  
828 Milligan, D.W., 2001, Pre-emptive Oral Ribavirin Therapy of  
829 Paramyxovirus Infections after Haematopoietic Stem Cell  
830 Transplantation: A Pilot Study. Bone Marrow Transplantation 28, 759-  
831 763.
- 832 Clark, H.F., Lief, F.S., Lunger, P.D., Waters, D., Leloup, P., Foelsch, D.W.,  
833 Wyler, R.W., 1979, Fer de Lance Virus (FDLV): A Probable Paramyxovirus  
834 Isolated from a Reptile. Journal of General Virology 44, 405-418.
- 835 Folsch, D.W., Leloup, P., 1976, [Fatal endemic infection in a  
836 serpentarium. Diagnosis, treatment and preventive measures]. Tierarztl  
837 Prax 4, 527-536.
- 838 Franke, J., Batts, W.N., Ahne, W., Kurath, G., Winton, J.R., 2006,  
839 Sequence Motifs and Prokaryotic Expression of the Reptilian  
840 Paramyxovirus Fusion Protein. Archives of Virology 151, 449-464.
- 841 Franke, J., Essbauer, S., Ahne, W., Blahak, S., 2001, Identification and  
842 Molecular Characterisation of 18 Paramyxoviruses Isolated from Snakes.

- 843 Virus Research 80, 67-74.
- 844 Freeman, A.F., Jacobsohn, D.A., Shulman, S.T., Bellini, W.J., Jaggi, P., de  
845 Leon, G., Keating, G.F., Kim, F., Pachman, L.M., Kletzel, M., Duerst, R.E.,  
846 2004, A New Complication of Stem Cell Transplantation: Measles  
847 Inclusion Body Encephalitis. *Pediatrics* 114, 657-660.
- 848 Gravendyck, M., Ammermann, P., Marschang, R.E., Kaleta, E.F., 1998,  
849 Paramyxoviral and Reoviral Infections of Iguanas on Honduran Islands.  
850 *Journal of Wildlife Diseases* 34, 33-38.
- 851 Hernandez-Divers, S.J., 2006, Appendix A Reptile Viral Diseases -  
852 Summary Table, In: *Reptile Medicine and Surgery*. Elsevier, St. Louis, pp.  
853 1147-1158.
- 854 Homer, B.L., Sundberg, J.P., Gaskin, J.M., Schumacher, J., Jacobson, E.R.,  
855 1995, Immunoperoxidase Detection of Ophidian Paramyxovirus in Snake  
856 Lung Using A Polyclonal Antibody. *Journal of Veterinary Diagnostic*  
857 *Investigation* 7, 72-77.
- 858 Huchzermeyer, F., Gerdes, G., Putterill, J., 1994. Viruses and  
859 mycoplasmas from faeces of farmed Nile crocodiles. In: *Proceedings of*  
860 *the 12th Working Meeting of the Crocodile Specialist Group*, Gland,  
861 Switzerland, pp. 303-308.
- 862 Hyndman, T.H., Marschang, R.E., Wellehan, J.F., Jr., Nicholls, P.K., 2012a,  
863 Isolation and molecular identification of Sunshine virus, a novel  
864 paramyxovirus found in Australian snakes. *Infection, Genetics and*  
865 *Evolution* 12, 1436-1446.
- 866 Hyndman, T.H., Shilton, C.M., Doneley, R.J.T., Nicholls, P.K., 2012b,  
867 Sunshine virus in Australian pythons. *Veterinary Microbiology* 161, 77-  
868 87.
- 869 ICTV 2013. *Virus Taxonomy: 2011 Release* (International Committee on  
870 *Taxonomy of Viruses*).
- 871 Jackson, O.F., Needham, J.R., 1983, Rhinitis and Virus Antibody Titres in  
872 Chelonians. *Journal of Small Animal Practitioners* 24, 31-36.
- 873 Jacobson, E., Gaskin, J.M., Page, D., Iverson, W.O., Johnson, J.W., 1981,  
874 Illness Associated with Paramyxo-like Virus Infection in a Zoologic  
875 Collection of Snakes. *Journal of the American Veterinary Medical*  
876 *Association* 179, 1227-1230.
- 877 Jacobson, E.R., 2007, *Viruses and Viral Diseases of Reptiles*, In:  
878 *Infectious Diseases and Pathology of Reptiles*. Taylor & Francis Group,  
879 LLC, Florida, pp. 395-460.

- 880 Jacobson, E.R., Adams, H.P., Geisbert, T.W., Tucker, S.J., Hall, B.J.,  
881 Homer, B.L., 1997, Pulmonary Lesions in Experimental Ophidian  
882 Paramyxovirus Pneumonia of Aruba Island Rattlesnakes, *Crotalus*  
883 *unicolor*. *Veterinary Pathology* 34, 450-459.
- 884 Jacobson, E.R., Flanagan, J.P., Rideout, B.R., Ramsay, E.C., Morris, P.,  
885 1999, Ophidian Paramyxovirus - Roundtable Forum. *Journal of*  
886 *Herpetological Medicine and Surgery* 9, 15-22.
- 887 Jacobson, E.R., Gaskin, J.M., Flanagan, J.P., Odum, R.A., 1991, Antibody  
888 Responses of Western Diamondback Rattlesnakes (*Crotalus atrox*) to  
889 Inactivated Ophidian Paramyxovirus Vaccines. *Journal of Zoo and*  
890 *Wildlife Medicine* 22, 184-190.
- 891 Jacobson, E.R., Gaskin, J.M., Simpson, C.F., Terrell, T.G., 1980,  
892 Paramyxo-like Virus Infection in a Rock Rattlesnake. *Journal of the*  
893 *American Veterinary Medical Association* 177, 796-799.
- 894 Jacobson, E.R., Gaskin, J.M., Wells, S., Bowler, K., Schumacher, J., 1992,  
895 Epizootic of Ophidian Paramyxovirus in a Zoological Collection:  
896 Pathological, Microbiological, and Serological Findings. *Journal of Zoo*  
897 *and Wildlife Medicine* 23, 318-327.
- 898 Jacobson, E.R., Origgi, F., Pessier, A.P., Lamirande, E.W., Walker, I.,  
899 Whitaker, B., Stalis, I.H., Nordhausen, R., Owens, J.W., Nichols, D.K.,  
900 Heard, D., Homer, B., 2001, Paramyxovirus Infection in Caiman Lizards  
901 (*Draecena guianensis*). *Journal of Veterinary Diagnostic Investigation* 13,  
902 143-151.
- 903 Jacobson, E.R., Origgi, F.C., 2007, Serodiagnostics, In: *Infectious*  
904 *Diseases and Pathology of Reptiles*. Taylor & Francis Group, LLC, Florida,  
905 pp. 381-394.
- 906 Jacobson, E.R., Samuelson, D.A., 2007, Identifying Reptile Pathogens  
907 Using Electron Microscopy, In: *Infectious Diseases and Pathology of*  
908 *Reptiles*. Taylor & Francis Group, LLC, Florida, pp. 299-350.
- 909 Junqueira de Azevedo, I.d.L.M., Prieto da Silva, A.R.B., Carmona, E., Lee  
910 Ho, P., 2001, Characterisation of a Paramyxovirus from a Fer de Lance  
911 Viper (*Bothrops jararaca*): partial nucleotide sequence of the Putative  
912 Fusion Protein. *Archives of Virology* 146, 51-57.
- 913 Kania, S.A., Kennedy, M., Nowlin, N., Diderrich, V., Ramsay, E., 2000.  
914 Development of an enzyme linked immunosorbent assay for the  
915 diagnosis of ophidian paramyxovirus. In: *Proceedings of the European*  
916 *Association of Zoo and Wildlife Veterinarians, Third Scientific Meeting,*  
917 *Paris, France, pp. 71-73.*
- 918 Kik, M.J.L., Van Zon, R.T., Van Asten, F.J.A.M., 2004. Retrospective

- 919 Studies on Ophidian Paramyxovirus Infection in Snakes in the  
920 Netherlands Using the Polymerase Chain Reaction (PCR). Preliminary  
921 Results. In: Proceedings of the 7th International Symposium on  
922 Pathology and Medicine in Reptiles and Amphibians, Berlin, pp. 43-48.
- 923 Kindermann, J., Kubber-Heiss, A., Kerschbaumer, P., Nowotny, N., 2001,  
924 Phylogenetic Analysis of the L and HN Gene of Ophidian  
925 Paramyxoviruses. Archives of Virology 146, 1021-1035.
- 926 Kolesnikovas, C.K.M., Grego, K.F., Rameh de Albuquerque, L.C.,  
927 Jacobson, E.R., Monezi, T.A., Mehnert, D.U., Catao-Dias, J.L., 2006,  
928 Ophidian Paramyxovirus in Brazilian Vipers (*Bothrops alternatus*).  
929 Veterinary Record 159, 390-392.
- 930 Kummrow, M., Lopez, J., Hatt, J., Dorrestein, G.M., 2004. Disease-risk  
931 Assessment for Free-ranging Geckoes (*Gecko monarchus*, *Gehyra*  
932 *mutilata*) in a Captive Reptile Stock. In: Proceedings of the AAZV, AAWV,  
933 WDA Joint Conference, San Diego, CA, p. 591.
- 934 Kurath, G. 2009. Reptile paramyxoviruses: 1 new species and new genus,  
935 Ferlavirus.
- 936 Kurath, G., Batts, W.N., Ahne, W., Winton, J.R., 2004, Complete Genome  
937 Sequence of Fer-de-Lance Virus Reveals a Novel Gene in Reptilian  
938 Paramyxoviruses. Journal of Virology 78, 2045-2056.
- 939 Lloyd, C., Manvell, R., Drury, S., Sainsbury, A.W., 2005, Seroprevalence  
940 and Significance of Paramyxovirus Titres in a Zoological Collection of  
941 Lizards. Veterinary Record 156, 578-580.
- 942 Lloyd, M.L., Flanagan, J., 1991. Recent Developments in Ophidian  
943 Paramyxovirus Research and Recommendations on Control. In:  
944 Proceedings of the American Association of Zoo Veterinarians, pp. 148-  
945 153.
- 946 Lunger, P.D., Clark, H.F., 1978, Reptilia-Related Viruses. Advances in  
947 Virus Research 23, 159-204.
- 948 Manvell, R.J., Drury, S.E., Geach, M., Lewis, J.C.M., 2000, Isolation of  
949 Ophidian Paramyxovirus type 7 from a Reticulated Python in the UK.  
950 Veterinary Record 147, 696.
- 951 Marschang, R.E., 2011, Viruses Infecting Reptiles. Viruses 3, 2087-2126.
- 952 Marschang, R.E., Chitty, J., 2004, Infectious Diseases, In: BSAVA Manual  
953 of Reptiles. British Small Animal Veterinary Association, Gloucester, pp.  
954 330-345.
- 955 Marschang, R.E., Donahoe, S., Manvell, R., Lemos-Espinal, J., 2002,



- 956 Paramyxovirus and Reovirus Infections in Wild-caught Mexican Lizards  
957 (*Xenosaurus* and *Abronia* sp.). *Journal of Zoo and Wildlife Medicine* 33,  
958 317-321.
- 959 Marschang, R.E., Papp, T., Frost, J.W., 2009, Comparison of  
960 paramyxovirus isolates from snakes, lizards and a tortoise. *Virus*  
961 *Research* 144, 272-279.
- 962 Mayr, A., Franke, J., Ahne, W., 2000, Adaptation of Reptilian  
963 Paramyxovirus to Mammalian Cells (Vero Cells). *Journal of Veterinary*  
964 *Medicine B* 47, 95-98.
- 965 Nogueira, M.F., Barrella, T.H., Da Silva, R.J., Lopes, C.A.M., Araujo Junior,  
966 J.P., 2002, Isolation of an Ophidian Paramyxovirus (OPMV) in a Captive  
967 Rattlesnake (*Crotalus durissus terrificus*) from Botucatu, Sao Paulo State,  
968 Brazil. *Journal of Venomous Animals and Toxins* 8, 168-173.
- 969 Oettle, E., Steytler, Y., Williams, M., 1990, High mortality in a tortoise  
970 colony. *South African Journal of Wildlife Research* 20, 21-25.
- 971 Oros, J., Sicilia, J., Torrent, A., Castro, P., Deniz, S., Arencibia, A.,  
972 Jacobson, E.R., Homer, B.L., 2001, Immunohistochemical Detection of  
973 Ophidian Paramyxovirus in Snakes in the Canary Islands. *Veterinary*  
974 *Record* 149, 21-23.
- 975 Papp, T., Pees, M., Schmidt, V., Marschang, R.E., 2010a, RT-PCR  
976 diagnosis followed by sequence characterization of paramyxoviruses in  
977 clinical samples from snakes reveals concurrent infections within  
978 populations and/or individuals. *Veterinary Microbiology* 144, 466-472.
- 979 Papp, T., Seybold, J., Marschang, R.E., 2010b, Paramyxovirus infection in  
980 a leopard tortoise (*Geochelone pardalis babcocki*) with respiratory  
981 disease. *Journal of Herpetological Medicine and Surgery* 20, 64-68.
- 982 Pasmans, F., Blahak, S., Martel, A., Pantchev, N., 2008, Introducing  
983 Reptiles into a Captive Collection: The Role of the Veterinarian. *The*  
984 *Veterinary Journal* 175, 53-68.
- 985 Potgieter, L.N.D., Sigler, R.E., Russell, R.G., 1987, Pneumonia in Ottoman  
986 Vipers (*Vipera xanthena xanthena*) Associated with a Parainfluenza 2-  
987 like Virus. *Journal of Wildlife Diseases* 23, 355-360.
- 988 Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J., Leonard, F.C.,  
989 2002, Paramyxoviridae, In: *Veterinary Microbiology and Microbial*  
990 *Disease*. Blackwell Science Ltd, Oxford, pp. 381-389.
- 991 Richter, G.A., Homer, B.L., Moyer, S.A., Williams, D.S., Scherba, G.,  
992 Tucker, S.J., Hall, B.J., Pederson, J.C., Jacobson, E.R., 1996,  
993 Characterisation of Paramyxoviruses Isolated from Three Snakes. *Virus*

- 994 Research 43, 77-83.
- 995 Ritchie, B., 2006, Virology, In: Medicine and Surgery of Reptiles.  
996 Elsevier, St. Louis, pp. 391-417.
- 997 Rösler, R., Abbas, M.D., Papp, T., Marschang, R.E., 2013, Detection of  
998 antibodies against paramyxoviruses in tortoises. Journal of Zoo and  
999 Wildlife Medicine In press.
- 1000 Sand, M.A., Latimer, K.S., Gregory, C.R., Rakich, P.M., Jacobson, E.R.,  
1001 Pennick, K.E., 2004, Molecular Diagnosis of Paramyxovirus Infection in  
1002 Snakes using Reverse Transcriptase-Polymerase Chain Reaction and  
1003 Complementary Deoxyribonucleic acid:Ribonucleic acid in situ  
1004 Hybridisation. Journal of Veterinary Diagnostic Investigation 16, 442-448.
- 1005 Watanabe, M., Mishin, V.P., Brown, S.A., Russell, C.J., Boyd, K., Babu,  
1006 Y.S., Taylor, G., Xiong, X., Yan, X., Portner, A., Alymova, I.V., 2009, Effect  
1007 of hemagglutinin-neuraminidase inhibitors BCX 2798 and BCX 2855 on  
1008 growth and pathogenicity of Sendai/human parainfluenza type 3  
1009 chimera virus in mice. Antimicrobial Agents and Chemotherapy 53,  
1010 3942-3951.
- 1011 Wellehan, J.F., Johnson, A.J., 2005, Reptile virology. The Veterinary  
1012 Clinics of North America. Exotic Animal Practice 8, 27.
- 1013 West, G., Garner, M., Raymond, J., Latimer, K.S., Nordhausen, R., 2001,  
1014 Meningoencephalitis in a Boelen's Python (*Morelia boeleni*) Associated  
1015 with Paramyxovirus Infection. Journal of Zoo and Wildlife Medicine 32,  
1016 360-365.
- 1017 Witte, A., 1993. Ein Beitrag zum Nachweis von Antikörpern gegen  
1018 Herpes- und Paramyxoviren bei verschiedenen Landschildkrötenarten.  
1019 Dissertation. Justus-Liebig-Universität Gießen.
- 1020 Yolken, R.H., Torsch, V.M., Berg, R., Murphy, B.R., Lee, Y.C., 1980,  
1021 Fluorometric Assay for Measurement of Viral Neuraminidase -  
1022 Application to the Rapid Detection of Influenza Virus in Nasal Wash  
1023 Specimens. The Journal of Infectious Diseases 142, 516-523.
- 1024 Zangger, N., Muller, M., Pagan, O., 1991. Viral Dermatitis in the Spur-  
1025 Tailed (*Testudo graeca*) and the Spur-Thighed Tortoise (*Testudo*  
1026 *hermanni*) in Switzerland. In: Proceedings of the 4th International  
1027 Colloquium on Pathology Medicine of Reptiles and Amphibians, Bad  
1028 Nauheim, Germany, pp. 25-29.  
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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 ferlavirus infection. Only the references  
 1033 that report on original data are included here.  
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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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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 <sup>§</sup>	
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)

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

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1038 Table 3 Histopathological changes seen associated with ferlaviral infection.  
1039 \*These findings were in snakes that were experimentally infected with  
1040 ferlavirus by endotracheal inoculation. <sup>§</sup>Brain was not examined histologically  
1041 in Jacobson et al. (1981), Potgieter et al. (1987), Jacobson et al. (1992), Homer  
1042 et al. (1995) or Jacobson et al. (1997). It is unclear whether the brain was  
1043 examined in Oros et al. (2001) and Kolesnikovas et al. (2006).  
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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 ferlavriral

1048 literature.

1049

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)

1050 Table 5 The methods that have been reported in the literature to isolate  
1051 paramyxoviruses from reptiles. VH2 = viper heart cells. TH1 = terrapene heart  
1052 cells. IgH2 = iguana heart cells. SPF = specific pathogen free. \*Only those  
1053 attempts where a paramyxovirus was successfully isolated using a different  
1054 biological substrate are mentioned. This is to exclude the possibility that the  
1055 unsuccessful attempt was because there was simply no virus in the inoculum.  
1056 However, the possibility that the virus did grow in these cells but was not  
1057 detected cannot be ruled out as further testing (e.g. polymerase chain reaction)  
1058 was not performed.  
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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 <sup>§</sup>
Syncytial/giant cell formation	(Abbas et al., 2011) <sup>ϕ</sup> (Jacobson et al., 1980; Jacobson et al., 1981; Blahak, 1995; Homer et al., 1995; Jacobson et al., 2001) (Jacobson et al., 1981)	(Jacobson et al., 2001) <sup>#</sup>	(Richter et al., 1996) <sup>1</sup> (Mayr et al., 2000) <sup>2</sup> (Jacobson et al., 2001) <sup>3</sup>	(Ahne et al., 1987; Ahne et al., 1999b)	(Ahne et al., 1999a) <sup>2</sup>	(Potgieter et al., 1987)*	(Clark et al., 1979)
Cytoplasmic inclusion bodies			(Mayr et al., 2000) <sup>2</sup>				
Cell lysis/monolayer destruction	(Abbas et al., 2011) <sup>ϕ</sup> (Jacobson et al., 1981; Blahak, 1995; Homer et al., 1995; Kolesnikovas et al., 2006) (Homer et al., 1995)		(Mayr et al., 2000) <sup>2</sup>	(Ahne et al., 1987; Ahne et al., 1999b)	(Ahne et al., 1999a) <sup>2</sup>	(Potgieter et al., 1987)*	(Clark et al., 1979)
Elongation of cell processes							
Cell vacuolisation					(Ahne et al., 1999a) <sup>2</sup>		
Cell rounding	(Kolesnikovas et al., 2006)						

1061 Table 6 The cytopathic effects of paramyxoviruses isolated from snakes and a  
 1062 lizard that have been described in the literature. <sup>§</sup>This isolate displayed CPE in  
 1063 nine reptilian and four mammalian cell lines after first being passaged through  
 1064 an embryonated snake egg. <sup>1</sup> Adapted from VH2 cells. <sup>2</sup> Adapted from IgH2 cells.  
 1065 <sup>3</sup> Adapted from TH1 cells. \* Undescribed cytopathic changes were also seen  
 1066 when this isolate was propagated in hamster kidney cells (BHK-21), swine  
 1067 testicular cells (ST), Vero cells and primary bovine turbinate cells (BTU). <sup>#</sup>This  
 1068 isolate was obtained from a caiman lizard (*Draecena guianensis*). <sup>ϕ</sup>Tissue  
 1069 homogenates were inoculated onto VH2 and IgH2 cells but cell line that the  
 1070 isolate was successful isolated with is not specified.  
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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	<a href="http://www.idexx.de">http://www.idexx.de</a>
Laboklin, Germany	<a href="http://www.laboklin.de/">http://www.laboklin.de/</a>
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.  
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