Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Virology

Rachel E. Marschang

Reptile virology has undergone rapid development over the past few decades. The use of next-generation sequencing (NGS) techniques has led to a rapid increase in the number of viruses described in reptiles. Additionally, transmission studies proving the role of specific viruses in disease processes have been carried out for a number of viral infections in various reptile species. Despite many advances, the factors involved in the development of viral disease in reptiles are, in many cases, unclear. Both host and viral factors are involved. Environmental factors, particularly temperature, have long been known to influence the immune system of reptiles. Increasing evidence suggests that other factors can play an important role in the outcome of viral infections in reptiles, including various pollutants and other infectious agents. Concurrent infections with several infectious agents, including multiple viruses, may occur, and the interactions and effects of these concurrent infections are not yet understood. This makes clinical evaluation of individual test results difficult and reinforces the need to consider multiple pathogens and critically evaluate results in these individual cases. It is critical to differentiate between infection and clinical disease caused by true viral pathogens in our reptile patients.

Many different methods are available for the diagnosis of viral infections in reptiles. These include methods for the detection of viruses, viral proteins, or viral genomes and serologic methods for the detection of an immune response to viral infection (generally restricted to antibody detection). Which method should be used in a specific situation depends on many different factors. These include the host species, clinical observations, time since infection, virus species, reason for testing, and test availability. Unfortunately, some of these factors such as virus species involved or time since infection are often unknown. None of the test systems available for reptile virology are fully standardized in that repeatability and reproducibility are not consistently studied. In addition, cross-reactivity and relationships between reptile viruses are not fully understood, and the specificity of some tests may therefore be lower than expected in some cases, or may be too high, and only a subset of related viruses may be detected using some assays. It is recommended to contact your laboratory before submitting samples (Table 30.1).

The viruses in this review are presented according to taxonomic position, with double-stranded (ds) DNA viruses (adenoviruses, including atadenoviruses, siadenoviruses, and "testadenoviruses"; herpesviruses; iridoviruses, including ranaviruses, invertebrate iridoviruses, and erythrocytic viruses; papillomaviruses; poxviruses) presented first, followed by single-stranded (ss) DNA viruses (circoviruses and a "tornovirus," parvoviruses), reverse transcribing DNA and RNA viruses (hepadnaviruses, retroviruses), dsRNA viruses (reoviruses), negative sense ssRNA viruses (members of the order *Mononegavirales*, including bornaviruses, paramyxoviruses [ferlaviruses], sunviruses, rhabdoviruses; arenaviruses; bunyaviruses), and positive sense ssRNA viruses (members of the order *Nidovirales* [corona- or toroviruses], members of the genus *Picornavirales* [picornaviruses], caliciviruses, flaviviruses, togaviruses) (Table 30.2).

ADENOVIRIDAE

Adenoviruses (AdVs) are the viruses most commonly identified in many squamate species, particularly bearded dragons (*Pogona* spp.), although they have also been detected in various chelonians and crocodilians. AdVs have a relatively high resistance to inactivation and can be difficult to disinfect. Current taxonomy of the family *Adenoviridae* suggests a coevolutionary lineage of the viruses with their hosts and additional host switches. Atadenoviruses have been hypothesized to have coevolved with squamate reptiles, while a new genus ("Testadenovirus") has been proposed to have coevolved with chelonian hosts.[1] This can be important in understanding the pathogenicity of the viruses, because viruses that have coevolved with their hosts may not cause disease or may only cause disease in immune-suppressed animals or in conjunction with other infectious agents or other factors, whereas switching of hosts may lead to severe disease and death. AdVs from crocodilians have not yet been characterized.

Adenoviruses in Squamates

AdVs appear to occur worldwide in captive populations, and antibodies to AdVs have been detected in wild boa constrictors (*Boa constrictor*) from Costa Rica (Marschang et al, Proc 6th Int Cong Vet Virol, 2003, p 152) and in wild-caught rattlesnakes from the United States.[3] All of the AdVs detected in squamates so far have belonged to the genus *Atadenovirus*. Clinical signs most commonly associated with AdV infection in squamates are gastrointestinal and neurologic, including anorexia, lethargy, wasting, head tilt, opisthotonus, and circling (Fig. 30.1) In individual cases, stomatitis, dermatitis, and pneumonia have also been described.[4] AdVs have also been detected in animals with no clinical signs of disease. Koch's postulates have been fulfilled for an AdV-induced hepatic necrosis in a boa constrictor.[5] Gross pathologic examination of animals that die with AdV infection can involve only the liver, which may be enlarged and have petechiae or pale areas scattered throughout. Histologically, these animals generally have hepatic necrosis. The intestine is also frequently affected, and documented changes include dilatation of the duodenum and hyperemia of the mucosa. Basophilic intranuclear inclusions are often seen in hepatocytes and enterocytes (Fig. 30.2), as well as in myocardial endothelial cells, renal epithelial cells, endocardium, epithelial cells of the lung, and glial and endothelial cells in the brain.[4]

An important observation in many squamate AdVs is their relative species specificity: specific lizard AdVs are mostly found in a single host species, most notably Agamid AdV-1 in bearded dragons. However,

TABLE 30.1 Laboratories to Contact for Additional Information and Reptile Virus Testing

Name	Website	Address for Submissions	Contact Person	Email Contact
North America				
UF Diagnostic Laboratories, College of Veterinary Medicine	labs.vetmed.ufl.edu/sample-requirements/zoo-med-infections/	April Childress, University of Florida, 2015 SW 16th Ave., Building 1017, Room V2-186, Gainesville, FL 32608	April Childress or James F.X. Wellehan	childressa@ufl.edu
Wildlife Epidemiology Lab, University of Illinois, College of Veterinary Medicine	vetmed.illinois.edu/wel	2001 S. Lincoln Ave., Urbana, IL 61802	Matt Allender	WildlifeEpi@vetmed.illinois.edu
Europe				
Laboklin	laboklin.com/?lang=en	Steubenstr. 4, 97688 Bad, Kissingen, Germany	Rachel E. Marschang	marschang@laboklin.com
Wildlife Diagnostic Service Center for Fish and Wildlife Medicine, Department of Infectious Diseases and Pathobiology, College of Veterinary Medicine, Vetsuisse Faculty, University of Bern	fiwi.vetsuisse.unibe.ch/	Länggassstrasse 122, 3001 Bern, Switzerland	Francesco Origgi	francesco.origgi@vetsuisse.unibe.ch
Chemisches- und Veterinäruntersuchungsamt Ostwestfalen-Lippe (CVUA-OWL) AöR	cvua-owl.de	Westerfeldstraße 1, 32758 Detmold, Germany	Silvia Blahak	silvia.blahak@cvua-owl.de
Australia				
The Hyndman Reptile Pathogen Lab	profiles.murdoch.edu.au/myprofile/tim-hyndman/	School of Veterinary & Life Sciences, South Street, Murdoch University, Western Australia 6150	Tim Hyndman	T.Hyndman@murdoch.edu.au
Asia				
Laboratory of Veterinary Pathology, School of Veterinary Medicine, Azabu University	www.azabu-u.ac.jp/english/laboratories/	1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan	Yumi Une	une@azabu-u.ac.jp

This table includes only laboratories that offer qualified consultations, regularly publish their methods in peer-reviewed publications, and are known to the author. There are many other labs available that offer qualified testing, but it is important that the practitioner understands what each test offered detects.

FIG 30.1 Bearded dragon (*Pogona vitticeps*) infected with an adenovirus. This lizard showed neurological signs including opisthotonus. (Courtesy of Jutta Wiechert.)

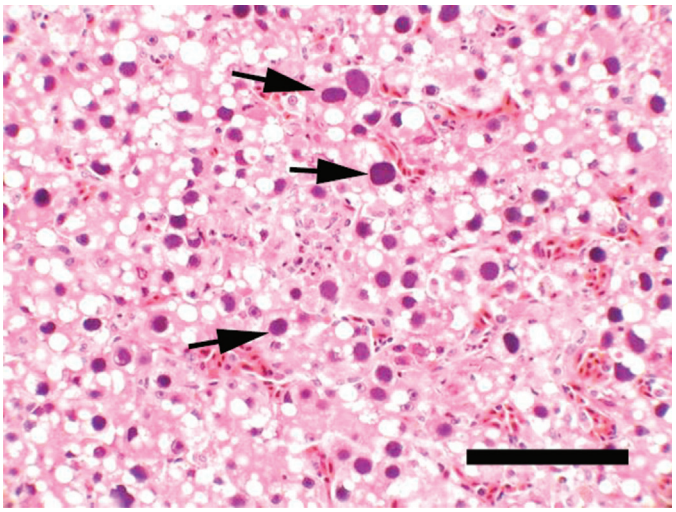

FIG 30.2 Liver of a bearded dragon (*Pogona vitticeps*). Note the large numbers of hepatocytes with large intranuclear basophilic or amphophilic inclusions (arrows). The hepatocytes also have moderate lipidosis. Hematoxylin and eosin; bar = 250 μm. (Courtesy of Michael M. Garner, Northwest ZooPath.)

TABLE 30.2 Virus Families Described in Select Groups of Reptiles (Including Endogenous Viruses)

	Chelonians					Squamates			
Virus Family	Ch1	Ch2	Ch3	Ch4	Ch5	S1	S2	S3	Crocodiles
Adenoviridae	X	X				X	X	X	X
Herpesviridae	X	X	X		X	X	X	X	X
Iridoviridae	X	X		X		X	X	X	
Papillomaviridae	X		X		X	X		X	
Poxviridae	X						X	X	X
Circoviridae		X	X			X			
Parvoviridae						X	X		
Hepadnaviridae		X		X		X	X		X
Retroviridae		X	X			X	X	X	X
Reoviridae	X					X	X	X	
Bornaviridae						X			
Paramyxoviridae	X					X	X	X	?
Sunviridae						X			
Rhabdoviridae		X		X		X		X	
Orthomyxoviridae									?
Arenaviridae						X			
Bunyaviridae				X				X	
Coronaviridae						X			?
Picornaviridae	X					X			
Caliciviridae						X			
Flaviviridae	X	X		X		X	X	X	X
Togaviridae	X	X		X		X	X	X	X

Ch1, Tortoises; *Ch2,* pond, box, and freshwater turtles; *Ch3,* sea turtles; *Ch4,* other Cryptodira; *Ch5,* side-necked turtles; *S1,* snakes; *S2,* Iguania (e.g., agamas, iguanas, chameleons); *S3,* other squamates; *?,* Some evidence of infection with these viruses in this group of reptiles.

there are a number of exceptions to this apparent rule, including the detection of a Helodermatid AdV-2-like virus in liver tissue of a western bearded dragon (*Pogona minor*) in Australia.[6] Serologic studies have also indicated that many different squamate reptiles can be infected with a variety of atadenoviruses.[3] AdV infections in squamates are also frequently found in conjunction with multiple other infections, including infections with parvo, reo, iridoviruses, coccidia, and microsporidia.

Adenoviruses in Chelonians

AdVs have only relatively recently been detected in several species of chelonians. In Travancore (Sulawesi) tortoises (*Indotestudo forstenii*), as well as in other tortoises in contact with the Travancore tortoises, infection was associated with severe systemic disease and a very high mortality rate (82%). Pathological findings in infected tortoises were multifocal hepatic necrosis, amphophilic to basophilic intranuclear inclusions and diffuse hepatic lipidosis, myeloid necrosis in bone marrow, and severe necrotizing enterocolitis. The virus detected in these tortoises differed distinctly from the AdVs characterized from squamates so far and was determined to belong in the *Siadenovirus* genus.[7] A single case of AdV infection has been reported in a leopard tortoise (*Stigmochelys pardalis*) that was also infected with a herpesvirus. This animal had biliverdinuria, wasting, and episodes of hemorrhages.[8] In Hungary, an AdV was detected in a box turtle with degeneration of liver cells, pronounced vacuolization of the cytoplasm, pyknosis of nuclei, and inclusion bodies in some hepatocytes.[9] Similar viruses have since been detected in a wider range of turtle and tortoise species in the United States and in Europe, including both healthy and diseased individuals. It has been proposed to classify these viruses in a new genus in the family *Adenoviridae*, "Testadenovirus."[1] There is some evidence that this lineage may have coevolved with chelonian hosts.

Adenoviruses in Crocodilians

Adenoviruses have been described in crocodilians, where they are mostly associated with liver disease in juvenile animals. The intestines, pancreas, and lungs are less commonly affected. Clinical signs that have been reported in infected animals have generally been limited to lethargy and anorexia. In chronically infected animals, hepatitis may lead to runting. Gross pathology may show swelling of the liver. Intestines may also be affected and show swelling. Histologically, intranuclear inclusions have been described mainly in hepatocytes and enterocytes.[10]

Identification of Adenovirus Infections

Virus Detection. A PCR targeting the DNA polymerase gene[11] has been frequently used to detect various AdVs in reptiles (Tables 30.3 and 30.4). It has been used on a wide range of samples and can detect AdVs described in squamates and chelonians. The PCR products should be sequenced, because this PCR can also detect AdVs of prey animals.[12] Recommended samples for the detection of AdVs in lizards and snakes are cloacal swabs (Fig. 30.3), liver, and intestine. Specific PCRs for the detection of reptilian AdVs have also been described. A real-time PCR has been developed for the detection of Agamid AdV-1.[13] This method will not detect other AdVs found in reptiles.

A number of AdVs from reptiles have been isolated in cell culture, which has facilitated further characterization of individual viruses. Snake AdVs have been isolated in several cases.[4] In lizards, AdVs have been isolated from helodermatid lizards,[12] and Agamid AdV-1 has been isolated on cells from bearded dragon embryos.[14]

Serology. Virus neutralization testing was first used for the detection of antibodies against a snake AdV (SAdV-1) (Marschang et al, Proc 6th Int Cong Vet Virol, 2003, p 152), demonstrating the presence of anti-AdV

TABLE 30.3 Select Viruses of Chelonians and Common Methods for Virus and Antibody Detection

Virus Family and Virus Genus	Virus Species or Strain	Host Species	Clinical Signs and/or Pathologic Changes Described in Infected Animals[1]	Diagnostic Samples in Order from Least to Most Invasive (if known, priority samples in bold)[2]	Virus Detection	Serology
Adenoviridae						
Siadenovirus[7]		Sulawesi tortoise (*Indotestudo forsteni*), Burmese star tortoise (*Geochelone platynota*)	Systemic disease, hepatic necrosis, necrotizing enterocolitis	Cloacal swabs, choanal swabs, **nasal flush**, oral/nasal mucosal tissue, plasma, **liver**, other tissues	PCR*	n.d.
"Testadenovirus"[1,9]		Various testudinid and emydid species	Hepatic and gastrointestinal lesions	Cloacal swabs, liver, intestine	PCR*	n.d.
Herpesviridae						
Scutavirus-like[33,54]	Lung, eye, and trachea disease (LETD) virus	Green sea turtles (*Chelonia mydas*)	Respiratory signs, buoyancy abnormalities, caseated material on the eyes, around the glottis, and in the trachea	Lung and trachea	PCR*, virus isolation	ELISA
Scutavirus[105]	*Chelonid alphaherpesvirus 5* (ChHV5), fibropapilloma-associated turtle HV (FPTHV)	Green sea turtles, loggerhead sea turtles, hawksbill turtles (*Eretmochelys imbricate*), Olive Ridley (*Lepidochelys olivacea*)	External and internal fibropapillomas	Fibropapillomas	PCR*	ELISA
Scutavirus-like[36]	LGRV	Loggerhead sea turtles (*Caretta caretta*)	Ulcers in the trachea, around the cloaca, and on the base of the phallus	Material from lesions	PCR*	n.d.
Scutavirus-like[36]	LOCV	Loggerhead sea turtles	Ulcers in the oral cavity, cutaneous plaques, pneumonia	Material from lesions	PCR*	n.d.
Scutavirus-like[39,41,60]	Testudinid HV1 (TeHV1)	Russian tortoise (*Testudo horsfieldii*), other testudinid spp.	Stomatitis, glossitis, rhinitis	**Oral swabs**, **tongue**, liver, brain, other tissues	PCR*, virus isolation	NT*, ELISA
Scutavirus-like[40,56]	TeHV2	Desert tortoise (*Gopherus agassizzii*)	Stomatitis, glossitis	**Tongue**, liver, other tissues	PCR*, rtPCR	ELISA
Scutavirus-like[41,60]	TeHV3	Many different species of Testudinidae	Stomatitis, glossitis, rhinitis, paralysis, incoordination	**Oral swabs, tongue**, liver, brain, other tissues	PCR*, virus isolation	NT*, ELISA
Scutavirus-like[42,43]	TeHV4	Bowsprit tortoise (*Chersina angulata*), leopard tortoise (*Stigmochelys pardalis*)	No clinical signs detected in first description, respiratory signs	Oral swab	PCR*	n.d.
Scutavirus-like[47,48]	Emydid HV 1	Eastern river cooter (*Pseudemys concinna concinna*), northern map turtle (*Graptemys geographica*), painted turtle (*Chrysemys picta*)	Hepatic necrosis, nasal discharge, pneumonia	Choanal/cloacal swabs, liver	PCR*	n.d.
Scutavirus-like[49]	Emydid HV 2	Spotted turtle (*Clemmys guttata*), bog turtle (*Glyptemys muhlenbergii*)	No disease detected	Choanal/cloacal swabs	PCR*	n.d.
Scutavirus-like[49]	Glyptemys HV1	Bog turtle (*Glyptemys muhlenbergii*)	No disease detected	Choanal/cloacal swabs	PCR*	n.d.
Scutavirus-like[49]	Glyptemys HV2	Wood turtle (*Glyptemys insculpta*)	No disease detected	Choanal/cloacal swabs	PCR*	n.d.

Scutavirus-like[50]	Terrapene HV1	Eastern box turtle (*Terrapene carolina carolina*)	Stomatitis, rhinitis, esophagitis, tracheobronchitis, pneumonia, gastritis, enterocolitis, splenic vasculitis, hepatitis, interstitial nephritis,	Oropharyngeal swabs, pharyngeal, and nasal mucosa	PCR*	n.d.
Scutavirus-like[51,57]	Terrapene HV2	Eastern box turtle (*Terrapene carolina carolina*)	Papillomas	Skin lesions	PCR*, rtPCR	n.d.
Scutavirus-like[53]	Pelomedusid HV 1	West African mud turtle (*Pelusios castaneus*)	No disease detected	Oral and cloacal swabs	PCR*	n.d.
Iridoviridae						
Ranavirus[67,76,77]	FV3-like, CMTV-like	Many different species of turtles and tortoises	Lethargy, anorexia, nasal discharge, conjunctivitis, subcutaneous cervical edema, ulcerative stomatitis, hepatitis, enteritis, pneumonia	Cloacal swabs, oral swabs, blood, **liver,** gastrointestinal tract	PCR*, rtPCR virus isolation	ELISA
Reoviridae						
Orthoreovirus[125,137]	Unnamed	Spur-thighed tortoise (*Testudo graeca*)	Stomatitis	Oral swabs, tongue	PCR*, virus isolation	NT
Paramyxoviridae						
Ferlavirus[146,153,154]		Spur-thighed tortoise (*Testudo graeca*), hermann's tortoise (*Testudo hermanni*), leopard tortoise (*Stigmochelys pardalis*)	Pneumonia, dermatitis	**Lung**, other tissues	RT-PCR*, virus isolation	HI*
Picornaviridae						
Torchivirus[139,179,184]	Called virus "x"	Many different species of Testudinae, most often spur-thighed tortoises (*Testudo graeca*)	Softening of the carapace, stomatitis, rhinitis, conjunctivitis, ascites, tubular vacuolization in the kidneys	**Oral swabs**, tongue, trachea, **intestine**, other tissues	RT-PCR*, virus isolation	NT
"Rafivirus"[180]		Travancore (Sulawesi) tortoise (*Indotestudo forstenii*)	Found together with a siadenovirus during an outbreak of severe systemic disease	Liver, kidney	RT-PCR	n.d.

*Method most commonly commercially available.

[1]A causative relationship between infection and disease has not always been proven and, in some cases, viruses may also have been detected in animals with no overt clinical signs of disease.

[2]In general, tissues with lesions should be included in diagnostic testing in sick animals.

CMTV, Common midwife toad virus; *ELISA,* enzyme-linked immunosorbent assay; *FV3, frog virus 3*; *HI,* hemagglutination inhibition test; *HV,* herpesvirus; *LETD,* lung, eye, and trachea disease; *LGRV,* loggerhead genital-respiratory HV; *LOCV,* loggerhead orocutaneous HV; *n.d.,* not described; *NT,* neutralization test; *PCR,* polymerase chain reaction; *rtPCR,* real-time PCR; *RT-PCR,* reverse-transcriptase PCR; *TeHV,* testudinid herpesvirus.

TABLE 30.4 Select Viruses of Squamate Reptiles and Common Methods for Virus and Antibody Detection

Virus Family and Virus Genus	Virus Species or Strain	Host Species	Clinical Signs and/or Pathologic Changes Described in Infected Animals[1]	Diagnostic Samples in Order from Least to Most Invasive (if known, priority samples in bold)[2]	Virus Detection	Serology
Adenoviridae						
Atadenovirus[3,11,13]	Multiple including *Snake atadenovirus A*, Agamid AdV-1	Many different species	Gastrointestinal and neurological signs (including anorexia, wasting, head tilt), hepatic necrosis, stomatitis, dermatitis, pneumonia	**Cloacal swabs, liver, intestine**, other tissues	PCR,* virus isolation Agamid AdV 1: rtPCR*	NT
Herpesviridae						
Unclassified[23-26]		Various snake and lizard species	Oral lesions, skin lesions (papilloma-like), hepatic necrosis, necrosis of endothelial cells, reduced venom production	Material from lesions, liver, intestine	PCR*	n.d.
Iridoviridae						
Ranavirus[64,65]	FV3-like, BIV-like, others	Various snake and lizard species	Oral lesions, hepatic necrosis, skin lesions	Oral/cloacal swabs, blood, skin, liver	PCR,* rtPCR, virus isolation	n.d.
Iridovirus (invertebrate iridoviruses, IIV)[95]	IIV6-like	Various lizard species, including agamid, chameleonid, and iguanid lizards	Skin lesions, anorexia, emaciation	Skin, kidney, liver	rtPCR,* PCR, virus isolation	n.d.
Unclassified: erythrocytic viruses[80,86,88]		Numerous species including bearded dragons (*Pogona vitticeps*), Iberian rock lizards (*Iberolacerta monticola*), and ribbon snakes (*Thamnophis sauritus sackenii*)	Anemia, oral lesions, blepharospasm	Erythrocytes	PCR (generally diagnosed by histological examination and EM)	n.d.
Papillomaviridae						
Unclassified[90]		Various snakes and lizards	Papillomas	Skin lesions	PCR	n.d.
Parvoviridae						
Dependoparvovirus[110]	*Squamate dependoparvovirus 1*, others	Various snake and lizard spp.	Mostly found together with adenovirus infections, gastrointestinal, neurologic, and respiratory signs	**Intestine**, other tissues	PCR, cell culture	n.d.
Reoviridae						
Orthoreovirus[125]		Many different species	Pneumonia, enteropathy, hepatopathy, CNS signs, skin lesions	Oral and cloacal swabs, liver, kidney, intestine, lung, spleen, brain	RT-PCR,* virus isolation	NT

Bornaviridae						
Bornavirus (Hyndman T, personal communication, 2016)[141,142]	*Elapid 1 bornavirus*, others	Gaboon viper (*Bitis gabonica*), African garter snake (*Elapsoidea loveridgei*), various python spp.	Neurologic disease	**Oral-cloacal swabs**, blood, venom glands, **brain**, various other tissues	RT-PCR, NGS	n.d.
Paramyxoviridae						
Ferlavirus[146,149,151,15]		Many different snake and lizard species; most commonly viperid snakes	Pneumonia, CNS signs	Oral and cloacal swabs, **tracheal washes**, **lung**, brain, kidney, and other tissue	RT-PCR,* virus isolation	HI*
Sunviridae						
Sunshinevirus[160]	*Reptile sunshinevirus 1*	Australian pythons (including black-headed python [*Aspidites melanocephalus*], woma python [*A. ramsayi*],spotted python [*Aspidites maculosa*], and carpet python [*Morelia spilota* spp. and *M bredli*]), ball python (*Python regius*)	CNS signs, pneumonia	Oral-cloacal swabs, liver, kidney, lung, **brain**	RT-PCR, virus isolation	n.d.
Arenaviridae						
Reptarenavirus[166,167]	Multiple species	Various boa and python spp.	Inclusion body disease	Oral-esophageal swabs, blood, esophageal tonsils, **liver**, kidney, pancreas, **brain**, other tissues	RT-PCR,* virus isolation	n.d.
Nidovirales: Coronaviridae						
Unassigned[175]	*Ball python nidovirus 1*	Ball python (*Python regius*), Indian rock python (*Python molurus*), green tree python (*Morelia viridis*), carpet python (*Morelia spilota* spp.), boa constrictors (*Boa constrictor*)	Pneumonia, stomatitis/pharyngitis	**Oral swabs**, **tracheal washes**, blood, liver, **lung**, trachea, esophagus, spleen, heart, brain	RT-PCR*	n.d.

*Method most commonly commercially available.

[1]A causative relationship between infection and disease has not always been proven and in some cases, viruses may also have been detected in animals with no overt clinical signs of disease.

[2]In general, tissues with lesions should be included in diagnostic testing in sick animals.

BIV, Bohle iridovirus; *EM,* electron microscopy; *FV3, frog virus 3*; *HI,* hemagglutination inhibition test; *IBD,* inclusion body disease; *n.d.,* not described; *NT,* neutralization test; *PCR,* polymerase chain reaction; *rtPCR,* real-time PCR; *RT-PCR,* reverse-transcriptase PCR.

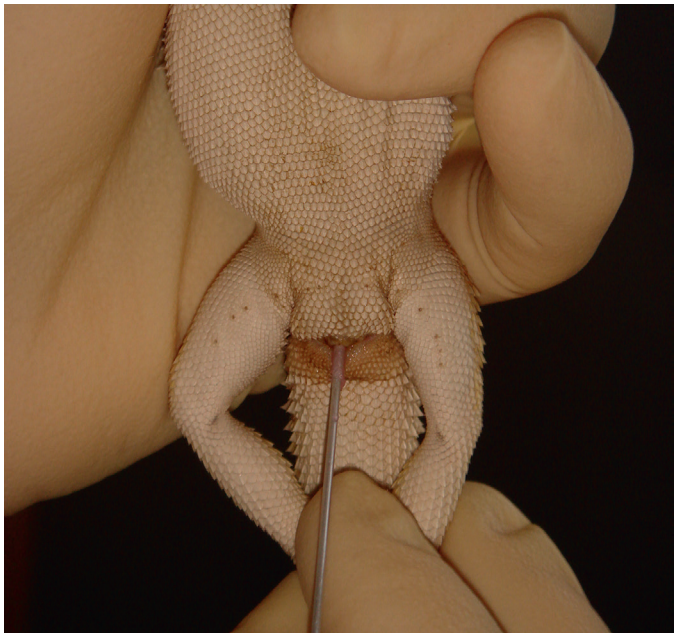

FIG 30.3 Bearded dragon (*Pogona vitticeps*). Cloacal swabs are recommended for the diagnosis of adenovirus infections in live animals.

antibodies in wild-caught snakes in Central America. In a study using Agamid AdV-1, Helodermatid AdV-1 and -2, and Snake AdV-1 and -2 in neutralization tests, antibodies against Agamid AdV-1 were most commonly found in agamid lizards, as well as in viperid and pythonid snakes, while antibodies against Helodermatid AdV-1 were most commonly found in iguanid lizards, and antibodies against Helodermatid AdV-2 were most commonly detected in helodermatid lizards.[3]

HERPESVIRIDAE

All of the reptilian HVs described so far appear to belong to the subfamily *Alphaherpesvirinae*. The genus *Scutavirus* has been created with the type species *Chelonid alphaherpesvirus 5* (chHV), also known as chelonid fibropapilloma-associated herpesvirus,[15] and herpesviruses from other chelonians have been shown to cluster with this genus.[16] It is considered probable that all vertebrates carry multiple HV species. In most cases, severe infection is only observed in very young or immunosuppressed animals or following infection of an alternative host.

Herpesviruses are relatively susceptible to disinfectants and are therefore easy to inactivate in the environment with the use of standard virucidal disinfectants. Studies on viral persistence in soil have shown that they are inactivated within about 2 weeks in the summer but may persist for longer at lower temperatures.[17] It is important to remember, though, that HV cause latent infections. Thus an HV-infected animal that survives initial infection must be considered a lifelong carrier.

In reptiles, HVs have been detected in lizards, snakes, chelonians, and crocodylians.[18] Infections are most often detected in various chelonian species, while detections in other species have been sporadic. In many, but not all, cases, infections have been associated with severe disease.

Herpesviruses in Squamates

In snakes, HVs have been detected in venom glands of various species, in some cases associated with decreased venom production,[19] and in a group of juvenile boa constrictors that died with hepatic necrosis.[20] In a disease outbreak among captive adult horned vipers (*Vipera ammodytes ammodytes*), all 71 horned vipers in the collection died within 2 weeks of developing lethargy, anorexia, and dyspnea. Common European vipers (*Vipera berus*) in the same collection were unaffected. Gross lesions in necropsied animals included petechiae and serosanguinous effusion throughout the body. Histology showed multiple foci of severe coagulative necrosis and necrosis of sinusoidal endothelial cells in the livers, myocardium, fat bodies, and spleens. Large acidophilic inclusion bodies were observed in the nuclei of endothelial cells in the liver.[21]

In lizards, a number of different HVs have been detected. HV-like particles, as well as particles resembling papova- and reoviruses, were detected by electron microscopy in papillomas of green lizards (*Lacerta viridis*).[22] Herpesviral DNA has also been detected by PCR in tissues from papillomas from green lizards.[23] A number of cases have been documented in which lizard HVs were associated with oral lesions in infected animals. Varanid HV 1 was detected in the oral mucosa and brain of emerald tree monitor lizards (*Varanus prasinus*) with proliferative stomatitis.[24] Three distinct HVs (gerrhosaurid HVs 1-3) were detected in Sudan plated lizards (*Gerrhosaurus major*) and a black-lined plated lizard (*Gerrhosaurus nigrolineatus*) with stomatitis.[25]

A few reports are available of HVs in lizards associated with lesions in the liver. Hepatitis and enteritis were diagnosed in juvenile monitor lizards that died acutely in the United States. Intranuclear inclusions were found in hepatocytes and enterocytes, and electron microscopy confirmed the presence of herpesvirus-like particles. The virus was shown to be closely related to Varanid herpesvirus 2 (GenBank accession No. AB189433), found in a monitor lizard in Japan (unpublished).[26]

Iguanid HV1 is the only lizard HV that has been isolated in cell culture from cell cultures derived from an infected green iguana (*Iguana iguana*). Transmission of the isolate to other lizards did not lead to the development of clinical signs.[27] In another case with a green iguana, HV-like particles were detected by electron microscopy in a lizard with hepatitis.[28]

Herpesviruses in Crocodilians

Several HVs have been reported in crocodilians. The first report was of the detection of HV-like particles in the skin of saltwater crocodiles (*Crocodylius porosus*) in Australia with a crust on the abdominal skin.[29] However, a direct link between the lesions and the HV detected could not be drawn, as the animals with lesions were shown to also have a poxvirus infection, as well as bacterial infections precipitated by biting. In the United States, an HV was detected in American alligators (*Alligator mississippiensis*) with lesions in the cloaca.[30] The virus was found to be very closely related to testudinid HV3, and the authors have suggested that it might have been a contaminant (GenBank accession number AY913769.1). Three different herpesviruses (Crocodyline HV1–3) were detected in saltwater and freshwater crocodiles from two large farms in Australia. The viruses were isolated in cell culture during an investigation into disease syndromes associated with conjunctivitis and pharyngitis, systemic lymphoid proliferation and encephalitis, and lymphonodular skin infiltrates in saltwater crocodiles and systemic lymphoid proliferation in freshwater crocodiles.[31]

Herpesviruses in Chelonians

Of the reptilian HVs, the chelonian HVs are most common and have been best characterized. HVs have been detected in members of the families Cheloniidae (sea turtles), Testudinidae (tortoises), Emydidae (pond turtles, box and freshwater turtles), Chelidae (Austro-American sideneck turtles), and Pelomedusidae (Afro-American sideneck turtles) so far.

In sea turtles, HV infections have been associated with skin lesions (gray patch disease); fibropapillomatosis; lung, eye, and trachea disease (LETD); loggerhead genital–respiratory HV (LGRV)-associated disease;

FIG 30.4 Green turtle (*Chelonia mydas*) with fibropapillomas.

and loggerhead orocutaneous HV (LOCV)-associated disease. Gray patch disease virus was one of the first HVs to be described in chelonians. It infects green sea turtles (*Chelonia mydas*). Aquaculture-reared, 2- to 3-month-old turtles appear to be most commonly affected. The virus was described by electron microscopy.[32]

Lung, eye, and trachea disease (LETD) has also been described in green sea turtles. Clinical signs associated with infection are gasping; harsh respiratory sounds; buoyancy abnormalities; inability to dive properly; and the presence of caseated material on the eyes, around the glottis, and within the trachea. Some of the infected turtles died after several weeks, while others became chronically ill. An HV (LETV) was isolated from diseased turtles in green sea turtle kidney cells.[33]

In sea turtles, fibropapillomatosis (Fig. 30.4) has been associated with HV infection and has been described in many different species of marine turtles, including green, loggerhead (*Caretta caretta*), Hawksbill (*Eretmochelys imbricata*), and olive ridley (*Lepidochelys olivacea*) sea turtles around the world. Infected turtles develop fibropapillomas, and individual or multiple tumors can occur externally all over the body. Internal tumors are also possible. The viral etiology has been tested by tumor transmission using cell-free tumor extracts.[34] The fibropapillomatosis HV has never been isolated in cell culture. Fibropapilloma-associated turtle HV (FPTHV) (also known as ChHV5) is considered the type species of the genus *Scutavirus*.[15] A study of geographically and genetically diverse FPTHVs indicated that these can be divided into groups, which cluster according to geographic origin, not host species.[35] Fibropapillomatosis has been treated by surgical removal of external fibropapillomas. These may recur, and the prognosis is poor in animals with extensive internal lesions or severely weakened animals.

Two HV-associated disease syndromes have been described in wild-caught loggerhead sea turtles, LGRV- and LOCV-associated disease. LGRV was associated with ulcers in the trachea, around the cloaca, and on the base of the phallus, while LOCV was associated with ulcers in the oral cavity and cutaneous plaques, which were covered with exudate and had an erythematous border, as well as with pneumonia.[36]

HV infections have been reported in many different species of tortoises. Clinical signs commonly associated with infections include rhinitis, conjunctivitis, stomatitis, and glossitis, which frequently develop into a diphtheroid-necrotizing stomatitis and glossitis (Fig. 30.5), with diphtheroid membranes covering parts of the oral cavity and extending down into the trachea and esophagus. Edema of the neck is a common sign. Affected animals are generally anorexic and lethargic. Animals that survive acute HV infection may develop central nervous system disorders, including paralysis or incoordination.[37] Hepatitis has also been described in affected animals. In a transmission study, spur-thighed tortoises inoculated with a tortoise HV either intramuscularly or intranasally developed disease signs consistent with HV infection.[38]

FIG 30.5 Russian tortoise (*Agrionemys horsfieldii*) with herpesvirus infection. Severe stomatitis with diphtheroid plaques visible throughout the oral cavity. (Courtesy of Volker Schmidt, Universität Leipzig, Leipzig, Germany.)

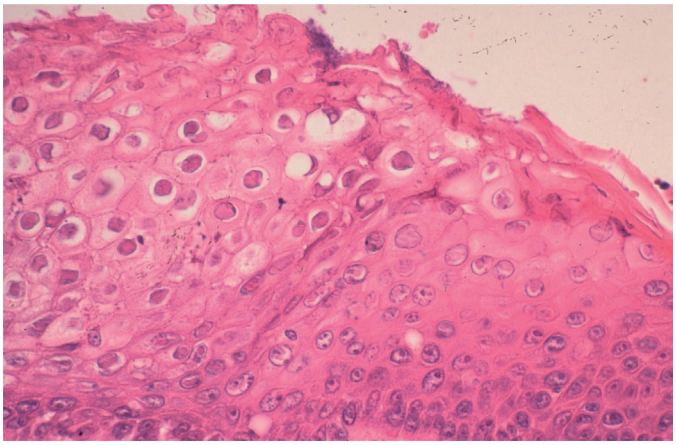

FIG 30.6 Herpesvirus infection in a tortoise (*Testudo hermanni*). Photomicrograph of the epithelium of the tongue. Ballooning degeneration is evident in numerous epithelial cells. Large intranuclear inclusions are also visible in several cells. H&E stain, ×400. (Courtesy of Horst Posthaus, Universität Bern, Berne, Switzerland.)

Development of disease and prognosis appear to depend both on the host species and on the virus involved.[37]

Histologically, HV infections in tortoises may be associated with eosinophilic or amphophilic intranuclear inclusions in infected tissues, most frequently in epithelial cells of the tongue, oral mucosa, and upper respiratory tract, as well as in the gastrointestinal tract (Fig. 30.6). Occasionally, inclusions can also be found in epithelial cells of the urinary tract, brain, liver, and spleen.[37]

There are currently four different HVs that have been shown to infect tortoises. They have been named testudinid HV1 to 4 (TeHV1 to 4).[18] TeHV1 was first detected in Russian tortoises (*Testudo horsfieldii*) and pancake tortoises (*Malacochersus tornieri*) in Japan.[39] Similar viruses have also been found in tortoises in Europe. Although they have been

detected in several different species, many cases so far have had direct contact with Russian tortoises. TeHV1 is associated with stomatitis in infected animals but does not appear to cause high morbidity or mortality. TeHV2 was described in a California desert tortoise (*Gopherus agassizii*) in the United States. The animal exhibited anorexia, lethargy, and yellow-white caseous plaques on the tongue and palate.[40] TeHV3 has been most frequently described in Mediterranean tortoises (Hermann's [*T. hermanni*], spur-thighed [*T. graeca*] and marginated [*T. marginata*] tortoises) and Russian tortoises in Europe. It has also been detected in tortoises in the United States and in northern Africa. This virus is associated with severe disease and high morbidity and mortality, particularly in Hermann's and Russian tortoises. Spur-thighed tortoises develop disease less frequently and appear to be able to survive and carry the infection.[41] TeHV4 was detected in a clinically healthy bowsprit tortoise (*Chersina angulata*) in a zoo in the United States.[42] A similar virus has since been detected in a leopard tortoise with respiratory disease that was co-infected with a mycoplasma in Europe.[43] Studies of the genomes of several distinct TeHV3 isolates have shown genetic differences between isolates, including deletions, which appear to influence the ability of the viruses to invade the brain and may therefore influence the virulence of individual strains.[16]

A number of reports are available on HV infection in water turtles. These were first described based on histological changes in Pacific pond turtles (*Actinemys* (*Clemmys*) *marmorata*), painted turtles (*Chrysemys picta*), and map turtles (*Graptemys* spp.). Clinical signs reported in affected animals include lethargy, anorexia, and subcutaneous edema. Characteristic necropsy findings include hepatomegaly and pulmonary edema. Areas of hepatic necrosis with the presence of intranuclear inclusion bodies in hepatocytes were reported. Inclusions have also been demonstrated in the spleen, lungs, kidneys, and pancreas.[44–46] More recent descriptions have included data on the genomes of the viruses detected. In all cases the detected viruses have clustered with other chelonian alphaherpesviruses. In one case in a captive freshwater turtle (*Pseudemys concinna concinna*) the animal died with no previous clinical signs. Histologic examination showed hepatic lipidosis with intranuclear inclusion bodies in hepatocytes.[47] A similar virus was also found in a northern map turtle (*Graptemys geographica*). The turtle had shown weakness and nasal discharge prior to death. Postmortem examination showed pneumonia, as well as hepatocellular and splenic necrosis, all associated with intranuclear inclusion bodies.[48] In a study screening wild bog turtles (*Glyptemys muhlenbergii*), wood turtles (*G. insculpta*), and spotted turtles (*Clemmy guttata*) in the northeastern United States, three different HVs were detected; 51.5% of the bog turtles sampled were HV positive. None of the animals in that study were clinically ill.[49] Herpesviruses have also been described in box turtles in the United States in association with stomatitis and papillomas.[50,51]

A single report is available of an HV infection in an Australian sideneck turtle, a captive Krefft's river turtle (*Emydura macquarii krefftii*). The animal showed ulcerative lesions of the skin and shell, and histopathology showed orthokeratotic hyperkeratosis with intranuclear inclusions in keratinocytes.[52] A single report also exists of an HV infection in African sideneck turtles (West African mud turtles [*Pelusios castaneus*]. The animals were imported into Germany from a farm in West Africa. A herpesvirus was detected in oral and cloacal swabs from clinically healthy animals by PCR.[53]

Identification of Herpesvirus Infections

Virus Detection. Detection of viral DNA by PCR is the most commonly used method for detecting HVs in infected reptiles (Tables 30.3 to 30.5). A PCR using degenerate primers in a nested format targeting a highly conserved portion of the DNA polymerase gene has been used to detect HVs in chelonians, squamates, and crocodylians.[54] Results from this PCR must be confirmed (e.g., by sequencing). Other PCRs have been described targeting specific TeHVs (e.g., only TeHV1 or only TeHV3).[41] A PCR for the differentiation of TeHV3 strains has been suggested.[55] A duplex PCR has been designed for the detection of CrHVs 1, 2, and 3 in formalin-fixed tissues from infected crocodiles.[31] Real-time, quantitative PCRs have also been developed for the detection of specific viruses in specific animal species, including TeHV2 in desert tortoises[56] and Terrapene HV1 in box turtles.[57] Virus isolation in cell culture has been used to detect LETV, TeHV1, and TeHV3. Samples for HV detection in chelonians should generally include tissues with lesions. For fibropapillomatosis, viral DNA can be detected in fibropapillomas removed from live or dead animals. In tortoises, HV DNA has been detected in oral swabs from live animals. Swabs should be taken from the base of the tongue and should include cellular material (Fig. 30.7). In dead tortoises, the tongue is generally considered the best tissue for virus detection. Esophagus, stomach, intestine, trachea, liver, and brain can also be helpful in virus detection. In water turtles, HV detection has most often been reported from the liver in dead animals. In crocodylians, CrHVs have been detected in conjunctiva, skin, liver, kidney, and mixed tissues of infected animals.

FIG 30.7 Hermann's tortoise (*Testudo hermanni*). Oral swabs can be used to diagnose several different viral infections in live tortoises, particularly herpes, picorna, and ranavirus infections.

Serology. Detection of antibodies against HVs is particularly important because HVs cause latent infections, therefore, any animal found to be serologically positive for HVs must be considered a lifelong carrier, even if the animal appears healthy. Serologic tests have been described for the detection of antibodies against FPTHV and LETV in sea turtles and against TeHV1 and TeHV3 in tortoises.[58–61] High seroprevalences were found in wild green sea turtles in Florida and in loggerhead turtles with an ELISA used for the detection of antibodies against glycoprotein H of an FPTHV.[58] Seropositivity did not correlate with clinical disease. However, testing for antibodies against this virus is not widely available. An ELISA has also been described for the detection of antibodies against LETV.[59]

Virus neutralization tests and ELISAs have been described for the detection of antibodies against TeHV1 and against TeHV3.[60,61] Virus neutralization testing with both TeHV1 and TeHV3 has shown that these viruses do not cross-react serologically; thus testing for antibodies against both is recommended in Europe. Detection of antibodies against these two viruses has been shown to depend on the tortoise species involved. Hermann's tortoises, which are particularly susceptible to HV infection and disease, do not often develop neutralizing antibodies after

TABLE 30.5 Select Viruses of Crocodilians and Common Methods for Virus and Antibody Detection

Virus Family and Virus Genus	Virus Species or Strain	Host Species	Clinical Signs and/or Pathologic Changes Described in Infected Animals[1]	Diagnostic Samples in Order from Least to Most Invasive (if known, priority samples in bold)[2]	Virus Detection	Serology
Herpesviridae[31]						
	Crocodyline HV 1–3	Saltwater crocodile (*Crocodylius porosus*), freshwater crocodile (*Crocodylus johnsoni*)	Conjunctivitis, pharyngitis, systemic lymphoid proliferation, encephalitis, lymphonodular skin infiltration	Conjunctiva, pharynx, skin, fat body, liver, kidney, lung, spleen, pancreas, intestine, thymus, heart, eye, brain	PCR, virus isolation	n.d.
Poxviridae						
Unclassified[96,97]	Caiman pox virus	Common caiman (*Caiman crocodilus*)	Gray-white papular skin lesions	Skin lesions	Histology and EM	n.d.
Crocodylidpoxvirus[101]	Nile crocodilepox virus (CRV) and others	*Crocodylus* spp.	Brownish wartlike skin lesions, in some cases, deeply penetrating skin lesions	Skin lesions	Histology and EM, PCR	n.d.
Flaviviridae						
Flavivirus[196,198,203,204]	*West Nile virus*	*Alligator mississippiensis, Crocodylus niloticus*	Neurologic signs (e.g., tremors, disorientation, opisthotonus), stomatitis, lymphohistiocytic proliferative cutaneous lesions	Cloacal swabs, **blood**, serum, **liver**, lung	RT-PCR*, virus isolation	NT, ELISA

*Method most commonly commercially available.
[1]A causative relationship between infection and disease has not always been proven, and, in some cases, viruses may also have been detected in animals with no overt clinical signs of disease.
[2]In general, tissues with lesions should be included in diagnostic testing in sick animals.
ELISA, Enzyme-linked immunosorbent assay; *n.d.,* not described; *NT,* neutralization test; *PCR,* polymerase chain reaction; *RT-PCR,* reverse-transcriptase PCR.

infection. In contrast, antibodies are frequently detected in spur-thighed tortoises that have been infected. An ELISA originally developed for the detection of antibodies against TeHV3 has also been adapted to detect antibodies against TeHV2 in California desert tortoises on the basis of putative serologic cross-reactivity between TeHV2 and 3.[40,62]

IRIDOVIRIDAE

Iridoviruses belonging to two (or three) genera have been detected in reptiles—ranaviruses, invertebrate iridoviruses and a putative additional genus in the family consisting of erythrocytic viruses. Although iridoviruses are enveloped, some viruses in this family (notably the ranaviruses) do not require their envelopes to be infectious. This makes them somewhat more resistant to disinfection (especially by organic solvents) than other enveloped viruses. Studies have shown that iridoviruses may be quite resistant to inactivation in water and soil at cool temperatures and may be able to survive over winter in regions with a temperate climate.[63] There is, however, evidence that ranaviruses can be inactivated in soil in the summer (at relatively high temperatures) within several weeks.[17] Under clean conditions, iridoviruses can be disinfected using standard virucidal disinfectants.

RANAVIRUSES

Ranaviruses have been increasingly shown to be important pathogens of ectothermic animals and are one of the major causes of global amphibian declines. Both phylogenetic studies and transmission studies indicate that some ranaviruses can be transmitted between reptiles, amphibians, and fish. Analysis of whole and partial genomes from reptilian ranaviruses indicates that several different species of amphibian ranaviruses, including viruses closely related to *Frog virus 3* (FV3), the type species of the genus *Ranavirus*, as well as *Bohle iridovirus* (BIV)-like, *common midwife toad virus* (CMTV)-like, and *European catfish virus* (ECV)-like viruses can infect reptiles.[64,65] Development of disease in infected animals is dependent on the virus strain, on the host species, and on environmental factors, particularly temperature. Some animals can become inapparent carriers, while in others, infection leads to severe disease and death.

Chelonians

In chelonians, ranaviral infection has been associated with lethargy, anorexia, nasal discharge, conjunctivitis, severe subcutaneous cervical edema, ulcerative stomatitis, and "red-neck disease" (Fig. 30.8). Infection induces an inflammatory response, and detection of an acute-phase protein response has been described.[66] Histologically, infected animals have been found to have hepatitis, enteritis, and pneumonia. Basophilic intracytoplasmic inclusions have in some cases been described in epithelial cells of the gastrointestinal tract and hepatocytes of infected animals, although inclusion bodies are not always detected. In a transmission study with box turtles (*Terrapene ornata ornata*) and red-eared sliders (*Trachemys scripta elegans*), intramuscular injection of a ranavirus led to disease, including lethargy, anorexia, ocular discharge, conjunctivitis, oral plaques, and death in some animals.[67] Studies on turtles that have survived ranavirus outbreaks have suggested that some animals may become persistently infected.[68] Other transmission studies have shown that environmental temperature can affect the development of clinical disease and death in infected turtles, with a higher mortality rate documented in infected red-eared sliders kept at 22°C than at 28°C.[69]

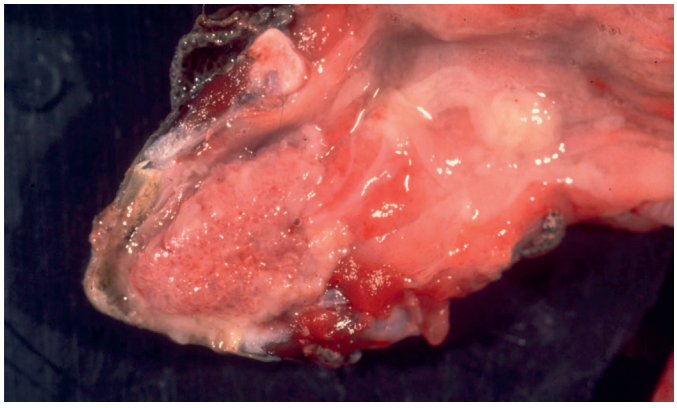

FIG 30.8 Hermann's tortoise (*Testudo hermanni*) infected with a ranavirus. Severe stomatitis. (Courtesy of Dr. Horst Posthaus, Universität Bern, Berne, Switzerland.)

Squamates

In the first description of infection in squamates, infected green tree pythons (*Morelia* [*Chondropython*] *viridis*) showed ulceration of the nasal mucosa, hepatic necrosis, and severe necrotizing inflammation of the pharyngeal submucosa.[70] In a giant leaf-tailed gecko (*Uroplatus fimbriatus*), infection was associated with granulomatous lesions in the tail and liver. In an Iberian mountain lizard (*Iberolacerta (Lacerta) monticola*), no overt disease was documented. That lizard was also infected with erythrocytic virus. In recent descriptions of ranavirus infections in various lizard species, variable skin lesions have been the most common lesion observed (Fig. 30.9). In many cases, infected lizards were from the international pet trade and had multiple other infections.[71–73]

Transmission. There is evidence that ranaviruses can be transmitted to reptiles by several different routes, including direct contact between animals, contact with contaminated water or pond sediment, and ingestion of infectious material, for example, from dead amphibians. In transmission studies, injection of virus into susceptible species has been successful,[67] but transmission via water has also been demonstrated.[74] Transmission by mosquitoes has also been proposed for FV3 in terrestrial turtles.[75]

Identification of Ranavirus Infections

Virus Detection. Molecular methods, including both conventional and real-time PCR, have been frequently used for the detection of ranaviruses in reptiles (see Tables 30.3 and 30.4). The gene most frequently targeted is the major capsid protein (MCP) gene.[76] A real-time PCR has been described for the detection of ranaviruses in eastern box turtles in the United States.[77] However, real-time PCRs can be very strain specific, and methods described so far in reptiles have generally only been able to detect FV3, not other ranavirus strains found in reptiles, thus the use of these methods could lead to false-negative results. Ranaviruses also grow well in cell culture and can be grown on a wide range of cell lines from reptiles, fish, mammals, and birds if the cells are kept at appropriate temperatures (below 32°C).

Samples for the detection of ranaviruses in chelonians should include liver and gastrointestinal tract. Spleen and kidney may also be virus positive.[67] In a transmission study viral DNA was detected in oral and cloacal swabs from intramuscularly infected red-eared sliders as early as 5 days postinoculation (p.i.) and until 26 days p.i. or until the animals died or were euthanized.[67] Oral and cloacal swabs and blood have all been used to detect ranavirus in naturally infected chelonians.[78,79]

Samples for ranavirus detection in squamates should include liver tissue and skin. A ranavirus has been detected in the blood of a live

FIG 30.9 (A) Ranavirus-infected Asian glass lizard (*Dopasia gracilis*). Skin lesions on the ventral surface of the body. An invertebrate iridovirus was also found in the skin of this animal. (B and C) Skin alterations observed in ranavirus-infected green anoles (*Anolis carolinensis*). An invertebrate iridovirus was also detected in these animals. (B) Multiple ulcera on the ventral abdominal surface. (C) Beige gray discoloration of the skin at the lateral abdomen. (D) Skin lesions in a ranavirus-infected central bearded dragon (*Pogona vitticeps*). (From Stöhr et al, 2013.)[72]

Iberian mountain lizard.[80] The optimal sample for the detection of ranaviruses in live squamates is unknown, but testing of mixed oral/cloacal swabs and blood is recommended. Skin biopsies can also be tested in cases with dermatologic signs.

Serology. An ELISA for the detection of anti-ranavirus antibodies in Burmese star tortoises, gopher tortoises (*Gopherus polyphemus*), and eastern box turtles has been developed.[81] Testing of various species with and without previous histories of ranaviral infection showed a low prevalence of anti-ranavirus antibodies in all cases.[81] This ELISA has also been adapted for the detection of anti-ranavirus antibodies in *Testudo* spp. in Europe.[78]

INVERTEBRATE IRIDOVIRUSES (IIV)

Until recently, viruses of the genus *Iridovirus* had only been described in invertebrates, in which they can cause lethal infections in a wide range of host species. These viruses have been repeatedly detected in various species of crickets sold as feed animals in the pet trade in Europe. Infected crickets show hypertrophy and bluish iridescence of the affected fat body cells.[82] In reptiles, IIVs have been isolated from the lung, liver, kidney, and intestine of two bearded dragons (*Pogona vitticeps*) and a chameleon (*Trioceros* (*Chamaeleo*) *quadricornis*) and from the skin of a frilled lizard (*Chlamydosaurus kingii*). The frilled lizard showed poxlike skin lesions, and one of the bearded dragons had pneumonia. The other lizards had died with nonspecific signs. A host-switch of this virus from prey insects to the predator lizards was postulated.[83] An IIV was isolated from several tissues of a high-casqued chameleon (*Trioceros* [*Chamaeleo*] *hoehnelii*) and successfully used to infect crickets of the species *Gryllus bimaculatus*.[84] IIV-like viruses have also been detected in multiple other lizards from various owners, as well as from crickets,[85] and in multiple lizards with skin lesions in which other viruses (e.g., ranaviruses) were detected (Fig. 30.9A–C).[71,72] The clinical significance of IIV infection in lizards is not always clear, because virus has been detected in clinically healthy animals, as well as in animals that were emaciated, had skin lesions, or died acutely.

Identification of IIV Infections

IIVs have been detected by virus isolation, conventional, and real-time PCRs (see Table 30.4). All of the methods used to detect IIVs in samples from reptiles can also be used to detect these viruses in feed insects.[85] IIVs grow on multiple cell lines from insects, reptiles, and mammals at 28°C. Because feed insects are often infected with IIVs, samples from the gastrointestinal tract, including oral and cloacal swabs in live animals,

should not be used for IIV detection in reptiles. Skin biopsies have been used for virus detection in live animals with skin lesions, and various internal tissues including liver have been successfully used in dead animals. IIVs are often detected together with other infectious agents, particularly adenoviruses (AdVs) in agamids.

ERYTHROCYTIC VIRUSES

Viral erythrocytic infections have been described in fish and amphibians as well as lizards, snakes, and turtles. These viruses have been preliminarily classified as iridoviruses, and recent studies based on partial sequences of the DNA polymerase gene have shown that erythrocytic viruses (also called erythrocytic necrosis viruses) of reptiles and fish probably belong to a new genus in the family *Iridoviridae*.[80,86,87] They have been hypothesized to be transmitted by invertebrates. These viruses are associated with inclusions in erythrocytes of infected animals (Fig. 30.10), and inclusions were originally believed to be parasites (*Toddia* and *Pirhemocyton* sp.). Clinical signs in infected animals range from apparently healthy to severe systemic disease and death. Lethargy and weight loss associated with anemia have been reported. In a study of an outbreak in bearded dragons, infection was associated with lesions on the tongue, reduced appetite and weight loss, lethargy, and blepharospasmus in several infected animals.[88] Morphological changes in infected erythrocytes, including anisocytosis and polychromasia, as well as hepatic necrosis have been documented in reptiles with erythrocytic necrosis virus infections. A transmission study conducted with the lizards *Iberolacerta* (*Lacerta*) *monticola* and *Lacerta schreiberi* showed that infection with these agents can become systemic and may lead to death if the animals are kept at suboptimal temperatures.[89]

Identification of Erythrocytic Viruses

Erythrocytic viruses have generally been diagnosed by detection of inclusions in erythrocytes in blood smears, followed by electron microscope confirmation of their etiology. Following partial characterization of the genomes of erythrocytic viruses from fish and reptiles, several PCRs have been published and may prove usefull for diagnostics.[87,88] Sampling for these viruses should always include peripheral blood as well as liver in dead animals.

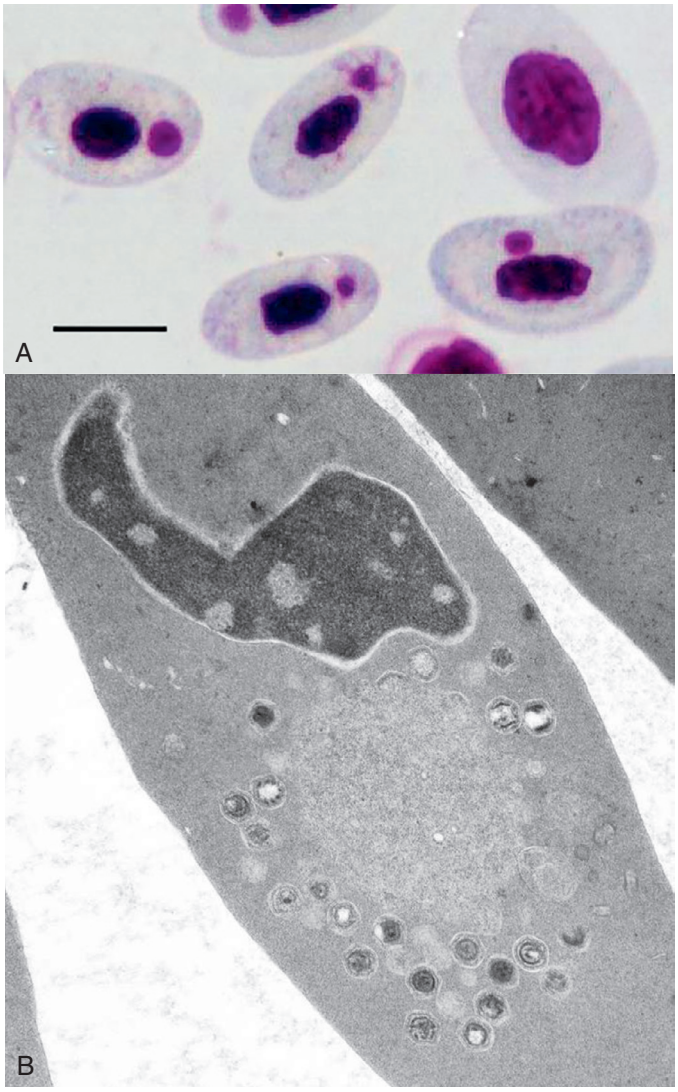

FIG 30.10 Iberian rock lizard (*Iberolacerta monticola*) erythrocytes with erythrocytic virus infection. (A) Erythrocytic necrosis virus has caused intracytoplasmic inclusions in multiple erythrocytes. H&E stain. (B) Transmission electron photomicrograph of the inclusion in an erythrocyte. The inclusions consist of viral precursors and viral particles in a crystalline array. (Courtesy of Antonio Pedro Alves de Matos, Curry Cabral Hospital, Lisbon, Portugal.)

PAPILLOMAVIRIDAE

Papilloma-like lesions (Fig. 30.11) have been repeatedly described in various reptiles. Their cause is not always known, and various viruses have been associated with such lesions. Papillomaviruses have only been associated with them in select cases. The papillomaviruses are highly host specific and tissue restricted. They generally cause benign tumors (warts, papillomas) in their natural host. Papillomaviruses are highly resistant to inactivation and can persist in the environment for long periods of time.

Papillomaviruses in Squamates

The first description of a papilloma-like virus (papovavirus) in reptiles was in wartlike skin lesions in a European green lizard. The virus was identified by electron microscopy based on morphologic characteristics. Herpeslike and reolike viruses were also identified in the lesions.[22] A papillomavirus has also been detected in a diamond python (*Morelia spilota spilota*) with multiple small black papillated and pedunculated skin proliferations, approximately 3 mm in diameter, as well as signs of respiratory disease.[90] The skin lesions were found to be a papilloma-like

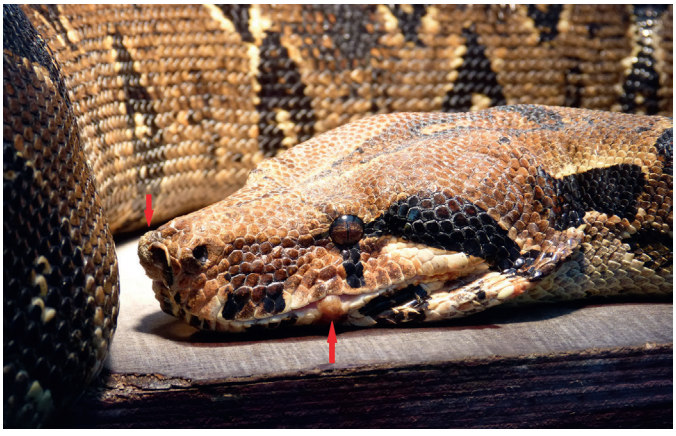

FIG 30.11 Wart-like lesions on the mouth and nares of a boa constrictor (*Boa constrictor*). This animal had inclusion body disease (IBD).

neoplasia, and inclusion bodies were found in some of the papillomas. A papillomavirus was identified in skin biopsies from this animal by PCR.[90]

Papillomaviruses in Chelonians

In chelonians, papillomaviruses have been described in Bolivian side-neck turtles (*Platemys platycephala*) with circular papular skin lesions that in some cases progressed to areas of necrosis. Viral particles were detected by electron microscopy in skin biopsies.[91] Papillomavirus-like particles were detected in a lung wash (but not in oral scrapings) by electron microscopy in a Russian tortoise with a history of stomatitis.[92] Lesions similar to those described in the Bolivian side-neck turtles were found in a loggerhead turtle and a green sea turtle.[93] The lesions resolved after several months. These two viruses (CcPV-1and CmPV-1) were shown to be distinct from one another and from previously described papillomaviruses.[93]

Identification of Papillomavirus Infections

Diagnosis of papillomavirus infections has been mostly by detection of viral particles in infected tissues via electron microscopy. The complete sequences of CcPV-1and CmPV-1 have been determined as has the complete sequence of a snake papillomavirus, so future development of diagnostic PCRs is possible.[94,95] However, these viruses are phylogenetically quite distant from one another, and nothing is known about genetic diversity of most reptilian papillomaviruses. Tissues for analysis should include affected skin.

POXVIRIDAE

Although poxviruses are enveloped, they are much more resistant to inactivation in the environment than other enveloped viruses and can remain infectious at ambient temperatures for months, especially when protected by cellular material (e.g., sloughed skin cells or blood). They are, however, inactivated by standard virucidal disinfection protocols.

The first report of poxvirus-associated disease in a reptile was in captive caimans (*Caiman crocodilus*) in the United States.[96] Similar cases have since been reported from caimans throughout the world.[97,98] Affected animals develop gray-white skin lesions on various parts of the body (Fig. 30.12).

Poxviruses have also been detected in Nile crocodiles (*Crocodylus niloticus*), as well as saltwater crocodiles (*Crocodylus porosus*) and freshwater crocodiles (*Crocodylus johnsoni*) in Australia.[99] Infected animals develop brownish wartlike skin lesions that can occur over the entire body (Fig. 30.13). Infection is associated with high morbidity but low mortality. A recent study of farmed saltwater crocodiles in Australia found that 40% to 45% of the examined animals were affected with mild cutaneous poxvirus infections.[100] An atypical form of crocodile pox has also been observed in Nile crocodiles. This virus was associated with deeply penetrating skin lesions in farmed crocodiles in Africa. A PCR was developed and used to amplify a portion of the genome of the virus associated with these lesions. Analysis of the PCR product showed that this virus was related to, but not identical with, crocodilepox virus (CRV).[101] Transmission is likely by contaminated water, possibly from infected wild animals as well as direct contact between animals. Infection by arthropod vectors as described for poxviruses of other animals may be possible.

Poxvirus infections have been detected in individual cases in other reptiles by electron microscopy. Papular skin lesions around the eyes of a Hermann's tortoise were found to contain poxlike viruses.[102] A flap-necked chameleon (*Chamaeleo dilepis*) in Tanzania was found to have two different types of intracytoplasmic inclusions in circulating monocytes. The inclusions were found to be caused by a chlamydialike organism and a poxlike virus.[103] A poxvirus infection in a tegu lizard was associated with brown papules on various parts of the body.[104]

Identification of Poxvirus Infections

Poxvirus infection in crocodilians has generally been diagnosed on the basis of the detection of intracytoplasmic inclusions in hypertrophic epithelial cells followed by electron microscopic demonstration of viral particles within these inclusions or by electron microscopic detection of viral particles in unfixed scrapings from lesions.[101,105] A PCR for the detection of CRV in scrapings from fresh lesions has also been described (see Table 30.5).[101]

CIRCOVIRIDAE AND TORNOVIRUS

Circoviruses are very resistant to inactivation in the environment and are very difficult to disinfect. A single circolike virus has been reported in macrophages of a painted turtle (*Chrysemys* sp.), with multifocal areas of necrosis in the spleen and liver. The virus was identified based on electron microscopy.[105] Studies of the genomes of snakes have shown that sequences of endogenous circoviruses can be found in these animals.[106]

A novel ssDNA virus with a circular genome (named sea turtle tornovirus 1, STTV1) was detected in two green sea turtles with fibropapillomatosis using metagenomics. Part of the genome was found

FIG 30.12 Caiman (*Caiman crocodilus*) with poxvirus infection. The skin lesions are covered with a white-gray crust. (Courtesy of Fritz W. Huchzermeyer, University of Pretoria, Onderstepoort, South Africa.)

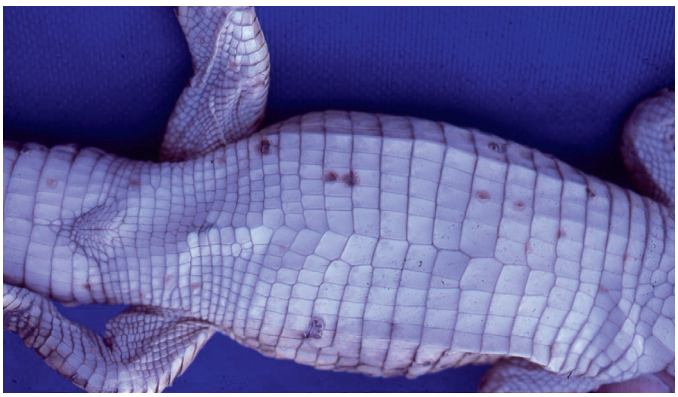

FIG 30.13 Nile crocodile (*Crocodylus niloticus*) infected with crocodile pox virus. Brownish lesions are seen on the abdominal skin. (Courtesy of Fritz W. Huchzermeyer, University of Pretoria, Onderstepoort, South Africa.)

to be distantly related to the circovirus *Chicken anemia virus*. Both of the infected turtles were severely afflicted with fibropapillomatosis. STTV1 was detected in the fibropapillomas, as well as in external swabs from the conjunctiva, oral cavity, cloaca, unaffected skin, and numerous internal tissues. The herpesvirus FPTHV was also detected in the fibropapillomas, but not in other tissues. STTV1 was also detected in leeches collected from one of the green sea turtles. It was hypothesized that STTV1 might affect the immune system of infected sea turtles or that it might be an opportunistic pathogen.[107]

PARVOVIRIDAE

The parvoviruses are highly resistant to inactivation in the environment and are therefore difficult to disinfect. They are particularly resistant to heat. The only classified reptile parvovirus, *Squamate dependoparvovirus 1*, is a species within the genus *Dependoparvovirus*.[108] Dependoparvoviruses are generally associated with a helper virus (adeno- or herpesviruses). In reptiles, parvolike viruses were first described by electron microscopy in the duodenum of a four-lined rat snake (*Elaphe quatuorlineata*) and of an Aesculapian snake (*Zamenis* [*Elaphe*] *longissimus*), both with gastrointestinal disease. Adenolike viruses were also detected in the duodenums of both and herpes- and picornalike viruses in the duodenum of one snake.[109] Co-infections of adenovirus- and parvoviruslike viruses have been described repeatedly from both snakes and lizards, with clinical signs including gastrointestinal disease, as well as neurologic signs and pneumonia.[18] A parvovirus but no helper virus was detected in a bearded dragon with malfunctioning ovaries and aberrant yolk formation, indicating that this virus is capable of independent replication.[110] Parvoviruses isolated in cell culture from a boa constrictor and a ball python (*Python regius*) were identical and were given the name serpentine adeno-associated virus (SAAV) or *Squamate dependoparvovirus 1*. It is unclear whether SAAV can replicate without a helper virus, as both isolates were obtained together with adenoviruses.[111]

Diagnosis of parvoviruses in reptiles has been by isolation in cell culture in various reptilian cell lines and, more recently, by PCR detection of viral DNA using degenerate primers targeting a portion of the genome of dependoparvoviruses.[110] Tissues from the intestinal tract have most often been used for virus detection, although mixed samples from various internal tissues have also tested positive in some cases.

HEPADNAVIRIDAE

Hepadnaviruses are enveloped viruses that include *Hepatitis B virus* (HBV). Integration of viral DNA into the host genome is rare but possible. Hepadnaviruses generally have narrow host specificity, although studies of virus evolution indicate frequent, recent host-switching, with incongruencies between host and virus phylogenies.[106] They are hepatotropic, and infection may be transient or persistent. Endogenous hepadnavirus sequences have been detected in the genomes of avian species and in rattlesnakes (*Crotalus mitchellii*) and the king cobra (*Ophiophagus hannah*).[106] In a transmission study, HBV-positive human serum was inoculated intramuscularly into yellow pond turtles (*Mauremys* [*Clemmys*] *mutica*) and Reeves' turtles (*Mauremys* [*Geoclemys*] *reevesii*). Viral antigene and antibodies against HBV were detected in most of the inoculated animals over a period of several weeks, and viral antigen and viral DNA were detectable in cultured liver cells from the inoculated animals. No signs of acute hepatitis were noted in the animals, although edematous changes in hepatocytes and minor cellular infiltration were reported and attributed to an inflammatory response.[112] HBV DNA has also been detected in a cloacal swab from a bearded dragon. The animal was from a reptile rescue in Germany and appeared clinically healthy at the time of sampling (Ball et al, Proc 9th Int Cong Vet Virol, 2012, p 60).

RETROVIRIDAE

Historically, retrovirus nomenclature was based on electron microscopy and classified members of the genera *Alpharetrovirus* and *Gammaretrovirus* as C-type viruses (assembly of immature capsids at the plasma membrane) and members of the genus *Betaretrovirus* as A-type particles (immature capsids) in the cytoplasm. A-type particles then budded with either B- or D-type morphology.[114] Retroviruses are widely distributed among vertebrates as exogenous infectious agents. Endogenous proviruses resulting from infection of germline cells also occur widely. Various retrovirus-like particles have been described in healthy snakes and in snakes with neoplasms.[18] Retroviral sequences have been detected in the genomes of many different reptiles. Systematic searches for sequences of murine leukemia–related retroviruses have shown that related viruses can be found in a wide range of animals, including reptiles, amphibians, birds, and mammals. In reptiles these viral sequences have been detected in the genomes of a number of chelonians, squamates, and in tuatara (*Sphenodon punctatus*).[115–117] A study on endogenous retroviral sequences from crocodilians showed that the genomes of several different species from different families in the order Crocodylia contain retrovirus sequences that are related to one another but highly divergent from other members of the *Retroviridae*, some of which appear to be species specific.[118] Retroviruses have been repeatedly isolated from boid snakes with inclusion body disease (IBD),[119,120] although IBD is now believed to be caused by arenaviruses. A retrovirus was isolated from an Indian rock python (*Python molurus*) that was kept together with IBD-positive pythons. Further study indicated that this is a highly expressed endogenous virus of pythons that is not associated with IBD. A similar virus was also identified in blood pythons (*Python curtus*).[121]

REOVIRIDAE

The family *Reoviridae* consists of non-enveloped viruses that are very stable in the environment. Reoviruses of reptiles that have been characterized so far have been classified in the genus *Orthoreovirus*. Viruses in this genus infect only vertebrates and are spread by the respiratory or fecal-oral route. Many orthoreovirus infections may cause no clinical signs of disease, or only mild upper respiratory tract illness and/or enteritis, while other species have been shown to cause significant and often fatal disease in avian and mammalian hosts. Five species have been classified in the genus *Orthoreovirus*, including *Reptilian orthoreovirus*. All but the *Mammalian orthoreovirus* isolates are fusogenic in cell culture.[122] Full genome sequencing of several reptilian othoreoviruses has shown that these may have originated from a common ancestor with fusogenic mammalian reoviruses and that they likely represent different species in the genus *Orthoreovirus*.[123,124] There is also evidence that individual reptilian reovirus strains are not host species specific.[125]

Reovirus infections have been relatively frequently described in reptiles. In lizards, reoviruses have been described by electron microscopy associated with papillomas in green lizards.[22] Other viruses were also detected in those lesions. Isolation of reoviruses from lizards has been described from green iguanas that died with no specific clinical signs in Germany[126] and from a spiny-tailed lizard (*Saara* [*Uromastyx*] *hardwickii*) in the United Kingdom that died during a disease outbreak associated with pneumonia.[127] An outbreak of reovirus-associated enteropathy and hepatopathy was described in leopard geckos (*Eublepharis macularius*) in the United States (Garner et al, Proc ARAV, 2009, p 82). Serologic tests have provided evidence of reovirus infections among other lizards as well, including wild-caught lizards in Central America.[129,130]

In snakes, reoviruses have been isolated from Chinese vipers (*Azemiops feae*) with enteritis, from ball pythons, emerald tree boas, and Aesculapian snakes.[18] Virus was isolated from the brain of a prairie rattlesnake (*Crotalus viridis*) with signs of central nervous system (CNS) disease, including incoordination, loss of proprioception, and convulsions.[131] A reovirus was detected in rough green snakes (*Opheodrys aestivus*) in the United States with a necrotizing hepatitis.[132] Rough green snakes imported into Hungary from the United States were also found to be infected with an orthoreovirus (Gál et al, Proc 8th Int Cong Vet Virol, 2009, p 228). Reoviruses were isolated from Moellendorff's rat snakes (*Orthriophis* [*Elaphe*] *moellendorffi*) and beauty snakes (*Orthriophis* [*Elaphe*] *taeniuris*) with fatal respiratory disease. The virus isolated from that outbreak was inoculated intratracheally, orally, and nasally into a black rat snake (*Pantherophis* [*Elaphe*] *obsoletus*). That snake died with pneumonia 26 days p.i. A reovirus was reisolated from the lungs of the dead snake.[134] Reoviruses were isolated from three boa constrictors with IBD in Germany, and a transmission study was carried out using one of these isolates. No specific disease or pathology was observed in the infected animals, although the virus was reisolated from the infected snakes up to 18 weeks p.i.[135] A reovirus was also detected repeatedly in a collection of corn snakes with increased mortality, as well as reported dyspnea, vomitus, and cachexia. A ferlavirus and an adenovirus were also detected in the same collection.[136]

Reovirus infections have been much less frequently described in tortoises. In one case a reovirus was isolated from a captive spur-thighed tortoise in Switzerland. The tortoise was cachectic and had a necrosis of the epithelium of the tongue.[137] A disease outbreak in spur-thighed tortoises in Germany with sudden softening of the carapax in juvenile animals, as well as an increased mortality rate, was associated with nematode, picornavirus, and reovirus infections in multiple animals (Blahak et al, Proc 33 Tagung der DVG Fachgruppe AVID 2014, pp 59-60).

Identification of Reovirus Infections

Virus Detection. Reoviruses of squamates are relatively easily isolated in cell culture (VH2 and IgH2), in which they cause the formation of giant syncytia. Virus has been isolated from oral and cloacal swabs from live snakes[136] and from liver, kidney, spleen, intestine, brain, and lung of dead animals. An RT-PCR has been described and used to detect and characterize reoviruses from various reptiles[125] but appears to be less sensitive than virus isolation in cell culture (see Tables 30.3 and 30.4).[136]

Serology. Virus neutralization tests have been used to detect antibodies against reoviruses in wild-caught green iguanas, Utila iguanas (*Ctenosaura bakeri*), spiny-tailed iguanas (*C. similis*), and knob-scaled lizards (*Xenosaurus grandis*).[129,130] In a study surveying wild-caught spur-thighed tortoises in Turkey, 4.9% of the animals tested had antibodies against a tortoise reovirus.[139] A study of the serologic cross-reactivity of six different reovirus isolates from lizards showed that at least three different serogroups exist; thus results of testing for antibodies using a virus neutralization test will depend on the virus used.[126]

BORNAVIRIDAE

Bornaviruses are neurotropic and do not cause the death of infected cells. Transmission may be direct or indirect, although bornaviruses of mammals do not appear to be readily transmissible between hosts. Bornaviruses are easily disinfected. In infected animals, virus persists in the host-cell nucleus. Endogenous bornalike elements (EBL) that have been integrated into the host genome have been found in the genomes of multiple species.[140] Both exogenous and endogenous bornaviruses have been detected in various reptile species in recent years. EBLs have been detected in the genomes of snakes, including speckled rattlesnakes (*Crotalus mitchellii*) and Burmese pythons (*Python bivittatus*).[106] Bornavirus N- and X/P-like sequences have been described in the venom glands (but not the genomic DNA) of a Gaboon viper (*Bitis gabonica*),[141] indicating that this is an exogenous virus, although no information about the clinical relevance of that virus (preliminarily named reptile bornavirus) is available. The full genome of a bornavirus, named Loveridge's garter snake virus 1, was sequenced from tissues of a museum specimen of an African garter snake (*Elapsoidea loveridgei*). It is unknown if any disease was associated with infection in that animal.[142] Recently, novel bornaviruses have been detected in pythons with neurologic disease in Australia. Virus detection by PCR has been successful in live animals from oral-cloacal swabs and whole blood, and from a range of tissues from dead animals. An oral-cloacal swab appears to be the priority sample from a live animal, and the brain appears to be the priority sample from a dead animal. Further research on these viruses and their clinical significance is ongoing, but preliminary results indicate that snakes can be persistently infected, and the correlation between clinical signs, histologic findings, and the presence of virus are not strong (Hyndman T, personal communication, 2016). These findings are not inconsistent with other bornaviruses known to be pathogenic.

PARAMYXOVIRIDAE: FERLAVIRUS

Paramyxoviruses (PMVs) are easily inactivated in the environment and by standard disinfectants. Some, but not all, members of this family have hemagglutinating and neuraminidase activity. The PMVs documented in reptiles belong to the genus *Ferlavirus*, which contains PMVs detected in snakes, lizards, and chelonians. These viruses have also been referred to as ophidian PMVs or reptilian PMVs. The genus *Ferlavirus* is named after the first PMV isolated from a snake, Fer-de-Lance virus (FDLV),[143] which is considered the type species of the genus.[108] All ferlaviruses hemagglutinate chicken red blood cells and hemagglutination inhibition assays have been used for serologic comparisons between isolates, as well as for diagnostic testing. Serologic studies have, however, shown that several ferlavirus strains can be distinguished from one another.[144,145] Isolates from squamates have been divided into three different genotypes (A, B, and C). A fourth genotype ("tortoise") has been isolated from a tortoise.[136,146] These genotypes do not appear to be host-species specific, and types A and B viruses have been found in a wide range of squamate hosts, as well as in chelonian hosts in individual cases.

Ferlaviruses in Squamates

The first PMV outbreak in snakes was described in 1976 in a serpentarium in Switzerland. During that outbreak, 87% of the snakes in one room died with dyspnea, opisthotonus, and apathy, followed by abnormal activity, mydriasis, and terminal convulsions.[147] Transmission appeared to be possible via aerosol, as well as via direct contact between snakes and fomites. Since that outbreak, ferlavirus outbreaks have been documented in numerous snake collections in North and South America and Europe, and the virus has been detected in wild-caught snakes from South America. Common clinical signs described in infected snakes include abnormal posturing, regurgitation, anorexia, head tremors, abnormal respiratory sounds, and exudate in the oral cavity (Fig. 30.14). In many cases, no clinical signs may be noticed, and infected animals may be found dead in their enclosures.[147,148] Severe disease has mostly been described in viperid snakes, but ferlaviruses have also been found in snakes from the families Colubridae, Elapidae, Boidae, and Pythonidae.[149] Koch's postulates have been fulfilled for pulmonary lesions associated with ferlavirus infection in Aruba Island rattlesnakes (*Crotalus durissus*

FIG 30.14 Bush vipers (*Atheris squamigera*) infected with ferlaviruses. (A) Dyspnea and bloody secretions in the oral cavity. (B) Opisthotonus. (Courtesy of Jutta Wiechert.)

unicolor). A ferlavirus isolated from several tissues of an Aruba Island rattlesnake that died of the infection was inoculated intratracheally into four Aruba Island rattlesnakes. Several snakes developed pulmonary signs, including blood in the lungs, trachea, and oral cavity. The two animals that were not euthanized earlier died 19 and 22 days p.i.[150] In another transmission study, genotypes A, B, and C ferlaviruses were inoculated intratracheally in corn snakes. That study demonstrated clear differences in pathogenicity between the three isolates used, with the type B virus causing the greatest pathology, with the lungs most severely affected.[151]

Gross abnormalities are most consistently found in the lungs of infected snakes. Changes include congestion and hemorrhage. Histologic findings often show proliferative interstitial pulmonary disease with proliferation and vacuolation of epithelial cells lining the faveoli. In rare cases, intracytoplasmic inclusions can be seen in lining epithelial cells.[148,150]

Although ferlaviruses are most commonly described in snakes, these viruses have also been detected in several species of lizard, including a spotted false monitor (*Callopistes maculatus*), an emerald tree monitor, a flathead knob-scaled lizard (*Xenosaurus platyceps*), and a group of Caiman lizards (*Dracaena guianensis*).[149] Ferlavirus infections in lizards have been associated primarily with pneumonia, although virus infections in clinically healthy lizards have been documented. The viruses do not appear to be host specific, and transmission between different species of snakes and lizards, as well as chelonians may be possible.

Ferlaviruses in Chelonians

Descriptions of ferlavirus infections in chelonians are rare. These infections have been associated with dermatitis (Zangger et al, Proc 4th Int Coll Path Med Rept Amphib, 1991, pp 25-29) and pneumonia.[146,153] In a single case, a PMV was isolated from a Hermann's tortoise with pneumonia. That virus was further characterized and found to be related to but distinct from the described ferlaviruses from snakes and lizards so far.[146] In another case, two distinct squamate ferlaviruses were detected in a leopard tortoise with pneumonia (Fig. 30.15). The detected viruses were each most closely related to ferlaviruses from snakes. However, it was unclear whether the virus was a causative agent, because no virus was detected in the lungs of the diseased animal.[153] Antibodies against ferlaviruses have been detected in several tortoise species.[154]

FIG 30.15 Leopard tortoise (*Stigmochelys pardalis*) with severe pneumonia. A ferlavirus was detected in this animal. (From Papp T, Seybold J, Marschang RE. Paramyxovirus infection in a leopard tortoise [*Geochelone pardalis babcocki*] with respiratory disease. *J Herp Med Surg* 2010;20:64–68. With permission.)

Identification of Ferlavirus Infections

Virus Detection. Ferlaviruses were first diagnosed in snakes by isolation in cell culture.[143] Virus isolation has since been used in many cases to detect these viruses in clinical samples from snakes and lizards. A number of RT-PCRs have also been described for the detection of ferlaviruses in reptiles. An RT-PCR targeting the conserved large polymerase (L) gene has been used to detect all of the ferlavirus genotypes detected to date.[146,155] In live animals, oral and cloacal swabs, as well as transtracheal washes, can be used as diagnostic samples. Oral and cloacal shedding can be inconsistent, and tracheal washes (Fig. 30.16) were found to be the most sensitive sample for ferlavirus detection in snakes during a transmission study, although virus was inoculated intratracheally in that study.[151] In dead animals, the highest viral load is found in the lung, and this tissue is recommended for testing (see Tables 30.3 and 30.4).[145,151] In addition, virus was frequently detected in intestine, pancreas, and brain.[151] The RT-PCR is not highly specific for ferlaviruses, and

FIG 30.16 Collection of a tracheal wash from a ball python (*Python regius*). Tracheal washes are the preferred sample for ferlavirus testing.

false-positive reactions have been shown to occur. For this reason, PCR products of the expected size (566 base pairs) should be sequenced. A direct comparison of virus isolation and virus detection by PCR showed that the PCR was more sensitive, although the highest sensitivity was achieved using a combination of both methods.[151]

Serology. Antibodies against ferlaviruses can be detected by hemagglutination inhibition (HI) assays using chicken red blood cells (see Tables 30.3 and 30.4). HI testing has been used repeatedly to detect exposure to ferlaviruses in squamates, including wild-caught snakes and lizards.[129,130,148] Serologic cross-reactivity among ferlaviruses and between ferlaviruses and other PMVs is not fully understood, and some (but not all) ferlaviruses have been shown to serologically cross-react with some avian PMVs.[145] Although serologic cross-reactivity exists between ferlaviruses, there is some indication that testing with different virus isolates will lead to different results. In a study comparing results of HI testing of plasma from eastern massasaugas *(Sistrurus catenatus catenatus)* from three different laboratories, different results were obtained from each; thus interpretation of results may be difficult.[156] Following transmission studies with three different ferlavirus genotypes (A, B, and C), clear differences were documented in the antibody titers detected against each virus, with lower or no titers detected in the group in which the highest pathogenicity was documented (infected with a genotype B virus), so that viral factors and pathogenicity may also play a role in development, titers, and persistence of antibodies.[157] Detection of anti-PMV antibodies indicates that an animal has been exposed to ferlaviruses or serologically related viruses. Persistence of ferlavirus infection in reptiles has not been recorded, but there is clinical evidence that this may occur. It is not known how long virus replication and shedding may persist after the development of HI antibodies.

SUNVIRIDAE: SUNSHINEVIRUS

A virus that is distantly related to the PMVs but differs distinctly from the ferlaviruses has been described in snakes in Australia. The virus has been named *Sunshinevirus* for the geographic location of the first isolation on the Sunshine coast of Australia. It has now been placed in a new family *Sunvirdae* in the order *Mononegavirales*. This virus was associated with neurorespiratory disease in Australian pythons and has 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*).[158] A single report exists of Sunshinevirus detection outside of Australia, in a ball python in Germany (Marschang et al, Proc ARAV, 2013, p 15). Clinical signs associated with infection may be similar to those reported for ferlavirus infections or be nonspecific (e.g., lethargy, inappetance). Histologically, the hindbrain white matter often exhibits spongiosis and gliosis. Neuronal necrosis has been described in severe cases. Some snakes may develop bronchointerstitial pneumonia.[160] Transmission is assumed to occur horizontally from oral and cloacal secretions, and there is evidence that the virus can also be transmitted vertically, although it may lead to the death of infected embryos.[161]

Sunshinevirus was originally detected by isolation in VH2 from diseased and contact snakes. A PCR has been described for the detection of this virus and appears to be more sensitive than isolation in cell culture. Virus detection has been carried out from oral and cloacal swabs in live animals. In dead animals, the virus is most often detected in the brain (see Table 30.4).[160]

RHABDOVIRIDAE

In reptiles, rhabdovirus infections have been documented based on both serology and virus detection. Little is known about the importance of rhabdoviruses in reptiles, but the rhabdoviruses detected in reptiles so far all appear to be arboviruses, some of which are also capable of infecting mammals. Antibodies against a vesicular stomatitis virus and Bahia Grande virus have been detected in wild-caught reptiles in Texas, while Charleville virus and Almpiwar virus have been isolated from lizards in Australia. Marco, Timbo, Chaco, and Sena Madureira viruses have been isolated from teiid lizards in South America as has another rhabdovirus from Caiman lizards.[162] Transmission studies with the rhabdovirus viral hemorrhagic septicemia virus (VHSV) and snapping turtles (*Chelydra serpentina*) and red-eared sliders have shown that aquatic turtles may be a vector for VHSV and that this virus may be able to infect turtles.[163] No clinical disease has been reported in association with these viruses in reptiles.

ARENAVIRIDAE

The family *Arenaviridae* has recently been divided into two genera, *Mammarenavirus* and *Reptarenavirus*. The genus *Reptarenavirus* was created to include arenaviruses detected in snakes and associated with IBD. These viruses have only been detected in snakes (no mammalian host), and their proteins differ from those of mammalian arenaviruses in several aspects.[164] Reptarenaviruses have been shown to be a genetically diverse group of viruses, including multiple species. It is even possible that arenaviruses of snakes may represent more than one genus.[165] Sequencing of full or partial genomes from a number of infected snakes has also shown that infection with multiple genotypes is common in captive infected snakes and that recombination and reassortment are common occurences.[166] Diversity is unequal between the genomic segments, with more L genotypes detected in infected animals than S genotypes. It has been hypothesized that human activity has led to an increased rate of viral evolution and that mixing of infected animals in captivity is a factor in virus diversity and in disease development.[165,166] The prevalence of reptarenaviruses in snakes in captivity, the existence of a possible reservoir hosts other than boid and/or pythonid snakes, and the genetic diversity of arenaviruses in the wild are not yet known.

INCLUSION BODY DISEASE (IBD)

IBD is a disease of snakes of the families Boidae and Pythonidae that has been described worldwide in captive snakes. The disease is characterized by the formation of intracytoplasmic inclusions in neurons and

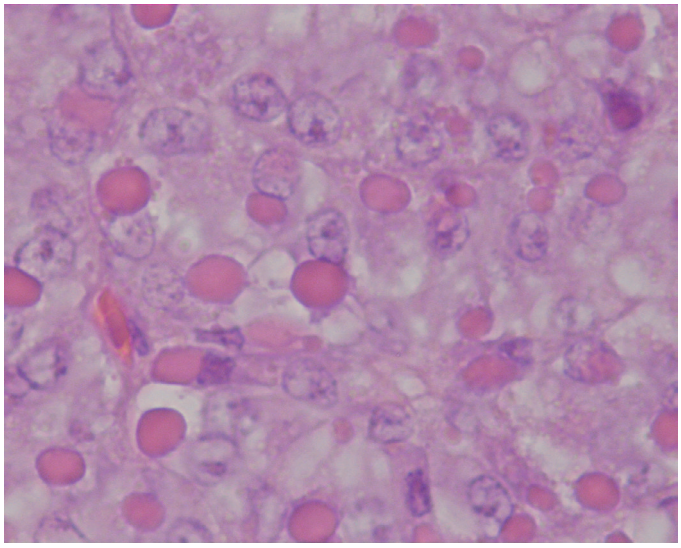

FIG 30.17 Multiple eosinophilic intracytoplasmic inclusion bodies in the pancreas of a *Boa constrictor* with IBD. H&E stain, 1000x. (Courtesy of Kim Heckers.)

in epithelial cells of various organs (Fig. 30.17). The etiology of IBD was long unknown, and various viruses have been proposed as possible causes of this disease over time.[119,120] Reptarenaviruses are now believed to be the cause of IBD.[167–169] In the first study describing the detection of these viruses, genetically variable arenaviruses were detected in 6 of 8 tested IBD-positive snakes and in none of 18 IBD-negative snakes by metagenomics and RT-PCR.[167] The inclusions are made up of a unique protein (inclusion body disease protein, IBDP), which appears to correspond to the reptarenaviral nucleoprotein.[167,168]

IBD was originally most commonly detected in Burmese pythons but is now most commonly diagnosed in boa constrictors.[105] Anecdotally, pythons tend to develop clinical diseases more quickly, while boas may remain inclusion-body positive but clinically healthy (or low morbidity, chronically diseased) for extended periods of time. The host range of IBD also includes the green anaconda (*Eunectes murinus*), yellow anaconda *(Eunectes notaeus)*, rainbow boa (*Epicrates cenchria*), Haitian boa (*Chilabothrus* [*Epicrates*] *striatus*), Madagascan boa (*Acrantophis madagascariensis*), annulated tree boa (*Corallus annulatus*), Indian rock python, reticulated python (*Malayopython* [*Python*] *reticulatus*), ball python, and carpet python.[167,170] Similar inclusions have also been reported in palm vipers (*Bothriechis marchi*) and an eastern king snake (*Lampropeltis getula*),[105,171] although it is not known if these were caused by the same virus. Clinical signs associated with IBD are variable and can range from subclinical carriers to severe neurologic disease and death. IBD is believed to cause immune suppression in affected animals, and many diverse clinical signs may result. Common signs in infected snakes include torticollis, disequilibrium, opisthotonus, inability to right itself, regurgitation, and flaccid paralysis (Fig. 30.18). Other signs that may also be observed include stomatitis and pneumonia. Skin lesions of various types have also been regularly observed in affected snakes. Lymphoproliferative disorders and round cell tumors have been described in infected snakes. Some snakes with IBD may die within weeks, but others may survive for extended periods of time. In some cases, boa constrictors have been described with inclusions but no apparent signs of clinical disease.

Studies on reptarenavirus-infected snakes with IBD have shown that infections with multiple genetically distinct viruses are very common.[165,166] As these viruses are believed to be immune suppressive, it has been hypothesized that reptarenaviruses may establish chronic infections in

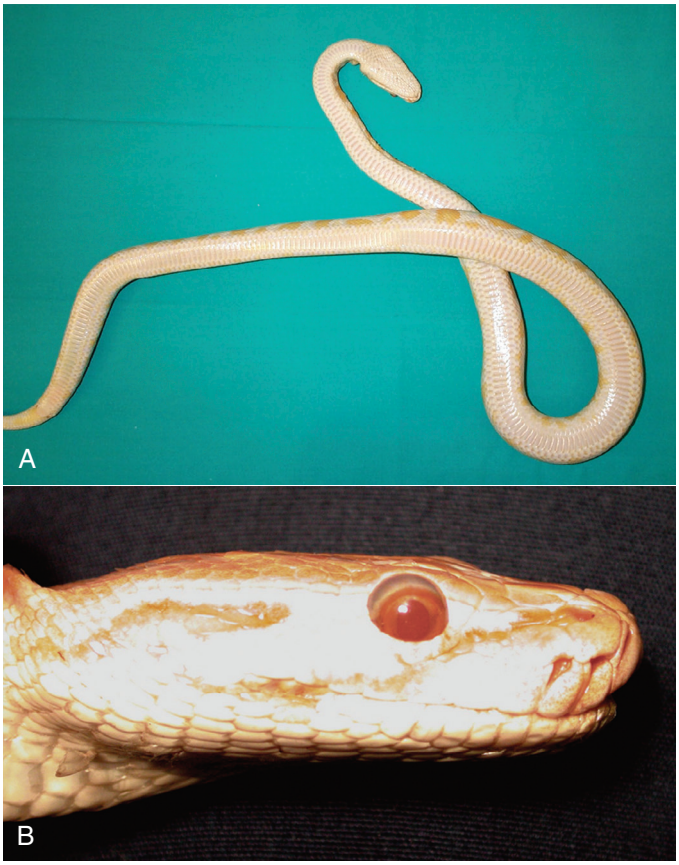

FIG 30.18 (A) Juvenile Burmese python (*Python molurus*) presented with acute onset of neurologic signs, including loss of the righting reflex and disorientation. (B) The same snake with mydriasis. (Courtesy of Jürgen Schumacher.)

snakes, possibly leading to acquired immunosuppression. Co- or superinfection of such snakes with another reptarenavirus could then result in amplified replication, with disease developing due to immunosuppression induced by the chronically infecting virus.[165]

Transmission of reptarenaviruses and development of IBD are not yet fully understood. Transmission from one animal to another appears to involve close contact over time, and cohabitation over a period of weeks or months has been demonstrated to lead to virus transmission.[166] Mites have been implicated as a possible vector,[170] and studies showing that a reptarenavirus can replicate in tick cells at 30°C support the hypothesis that these viruses may be transmitted by arthropod hosts and may even be arboviruses capable of replicating in arthropod and vertebrate hosts.[165] Transmission via biting among snakes and via aerosole have also been discussed. Vertical transmission appears to be possible, and reptarenaviruses have been detected in offspring from IBD-positive parents.[172]

Identification of IBD and Reptarenaviruses

Because IBD is defined by the presence of inclusion bodies in cells of affected snakes, cytology and histology remain important tools for IBD diagnostics. Typical eosinophilic to amphophilic intracytoplasmic inclusions in hematoxylin and eosin–stained tissue sections can be found in various tissues. The distribution of inclusions in tissues appears to depend on the host species. In pythons, inclusions are most commonly found in neurons within the central nervous system. In boa constrictors, they can also be found in glial cells, as well as in cells in the "esophageal tonsils" (Fig. 30.19), hepatocytes, pancreatic acinar cells, renal tubular

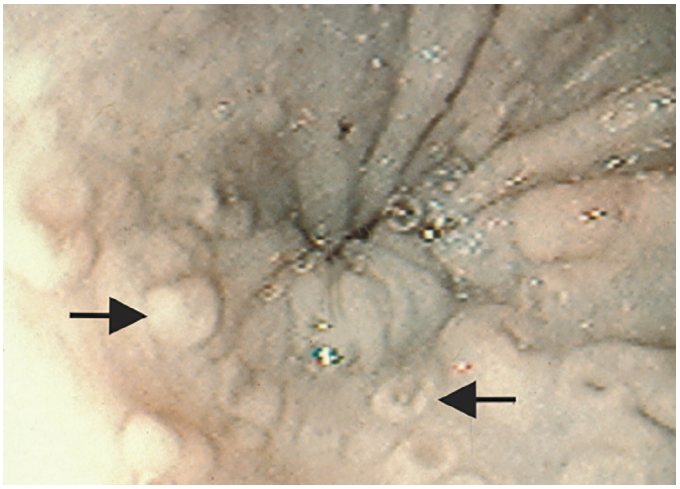

FIG 30.19 Endoscopic demonstration of prominent esophageal tonsils (arrows) in the distal esophagus of a boa constrictor (*Boa constrictor*) with inclusion body disease. (Courtesy of Jürgen Schumacher.)

epithelial cells, and epithelial cells lining the gastrointestinal and respiratory tracts.[170] In live boid snakes, inclusions can be detected in biopsies of the "esophageal tonsils," liver, and kidney. They can also be found in peripheral blood cells. Detection of inclusion bodies in live animals is much more common in boas than in pythons.

Virus detection via PCR has also become an important tool in the detection of infected animals and may be more sensitive than detection of inclusion bodies in many cases. A PCR targeting a portion of the glycoprotein gene has been described[166,167] and used for the detection of reptarenaviruses in live and dead snakes.[172] The glycoprotein gene is found on the S segment, in which less diversity has been detected than on the L segment, and this PCR has been shown to be able to detect diverse reptarenaviruses.[166] Samples used for virus detection in live snakes have included esophageal swabs/biopsies of esophageal tonsils, kidney, liver, gastric mucosa, and whole blood. In dead animals, brain is most often positive in pythons, while virus may be detectable in a wide range of tissues in boas (see Table 30.4). In some cases, animals that are arenavirus positive may not develop IBD. Persistence of virus in the brains of apparently clinically healthy animals has also been described.[172] Reptarenaviruses have also been isolated in cell culture in several cases, mostly in cell lines derived from boa constrictor kidney cells.[167,168]

BUNYAVIRIDAE

Bunyaviruses found in reptiles have all been arboviruses. A bunyavirus was isolated from the blood of a Texas soft-shelled turtle (*Apalone* [*Trionyx*] *spinifer emoryi*), which was wild-caught in Texas. The virus was isolated in suckling mice by intracerebral inoculation and caused 100% mortality in the mice. The virus was not further characterized but was found to serologically cross-react with Cache Valley and Tensaw viruses, both mosquito-borne viruses of the genus *Orthobunyavirus*.[173] Kowanyama virus, an unassigned member of the family *Bunyaviridae*, has been isolated from both mosquitoes and a skink (*Cryptoblepharus* [*Ablepharus*] *virgatus*) in Australia.[174] No disease has been described in association with bunyavirus infections in reptiles.

NIDOVIRALES: CORONAVIRIDAE

The order *Nidovirales* contains enveloped, positive-strand RNA viruses with the largest known RNA genomes. These viruses are relatively easily inactivated by chemical disinfectants. Nidoviruses have been identified in snakes, and these viruses appear to be most closely related to viruses in the family *Coronaviridae*, subfamily *Torovirinae*, and may represent a new genus in this subfamily.[175–177] Coronaviruses generally target the epithelia and are mostly associated with respiratory and gastrointestinal disease. A number of recent emerging viruses belong in this family.

In reptiles, nidoviruses have been found in snakes, particularly ball pythons in the United States and in Europe.[175,176] Nidoviruses have also been detected in Indian rock pythons, green tree pythons, a carpet python, and boa constrictors.[176,178] The most common manifestations of disease appear to be an interstitial proliferative pneumonia, but infected snakes have also been reported with a mild-to-severe tracheitis, mild-to-severe sinusitis/rhinitis, mild-to-moderate stomatitis/pharyngitis, esophagitis, mucous or caseous material in air passageways, and pulmonary hemorrhage.[175,176] Changes in other organ systems have also been detected in individual cases, including encephalitis, nephritis, nephrosis, multifocal dermatitis, salpingitis, hepatic lipidosis, conjunctivits, and pancreatic necrosis. It is unknown if changes in tissues outside the respiratory tract were related to the nidovirus infection.[175,176] Viruslike particles consisting of circular and elongated rod-shaped nucleocapsids were observed within pneumocytes of individual affected snakes by electron microscopy. Metagenomic sequencing was carried out on tissues from affected snakes, leading to detection of novel nidoviruses. No similar viruses were detected in nonaffected snakes.[175] Real-time and conventional PCRs have been used to detect nidoviruses in infected snakes. In live snakes, virus has most frequently been detected in oral swabs, although blood has also tested positive.[178] Tracheal washes may also be a sensitive sample for virus detection. Lung tissue has most often tested positive for nidoviruses, and the highest viral loads are detectable in respiratory tissues,[175] but virus has also been detected in the trachea, esophagus, liver, spleen, heart, and brain (see Table 30.4).[175,176]

PICORNAVIRIDAE

Two genetically distinct picornaviruses from reptiles have been characterized so far, both found in chelonians. They have been named torchiviruses (originally called topiviruses) and rafiviruses.[179,180] As unenveloped viruses, picornaviruses can be moderately difficult to disinfect. Studies of environmental persistence of torchiviruses show that these can persist for extended periods of time in water at cold temperatures, and inactivation at 37°C can take weeks. Inactivation in soil can take 3 weeks or more in the summer but significantly longer at lower temperatures.[17]

The first report of picornalike viruses in reptiles was based on the detection of 20- to 27-nm large viral particles in duodenal and splenic cells in a boa constrictor and in duodenal cells in an Aesculapian snake. Both snakes showed signs of gastrointestinal disease. An adenolike virus was also found in the duodenum and spleen of the boa constrictor, while parvo-, adeno-, and herpeslike viruses were detected in the duodenum of the Aesculapian snake.[109]

Picornalike viruses have been detected repeatedly by isolation in cell culture in a number of different tortoise species (Marschang et al, Proc ARAV, 2002, p 102). These viruses have been called Virus "X." They have most frequently been isolated from spur-thighed tortoises but have also been found in marginated tortoises, Hermann's tortoises, leopard tortoises, Egyptian tortoises (*Testudo kleinmanni*), and spider tortoises (*Pyxis arachnoides*). They have been associated with various signs of disease in infected animals, including softening of the carapace in young animals, diphtheroid-necrotizing stomatitis, rhinitis, conjunctivitis, and ascites (Fig. 30.20) but have also been isolated from clinically healthy animals.[137,182] There are no typical histologic lesions associated with infection. In numerous cases, tortoises infected with Virus "X"

FIG 30.20 Spur-thighed tortoise (*Testudo graeca*) with rhinitis. This animal was infected with a picornavirus (topivirus, "virus X"), as well as with a herpesvirus and *Mycoplasma*.

have also been shown to be infected with other pathogens, especially herpesviruses and *Mycoplasma* spp.[18] Sequencing of a large part of the genomes of isolates obtained from tortoises in Germany and Hungary over several years showed that all isolates were related to one another and belonged to the family *Picornaviridae*. They clearly form a new genus that has now been named "Torchivirus."[179] A transmission study in which juvenile spur-thighed and Hermann's tortoises were inoculated with a torchivirus isolate led to a decrease in bone density and tubular vacuolization in the kidneys (Paries et al, Proc iCARE 2015, p 351).

A genetically distinct picornavirus was detected in the spleen of a Travancore (Sulawesi) tortoise. The animal was from a group that died during a severe disease outbreak in which an adenovirus (Sulawesi tortoise adenovirus 1) was considered the causative agent.[7] The genus name "Rafivirus" was proposed for this new virus. The role of the detected picornavirus in the disease outbreak was unknown.[180]

Identification of Picornavirus Infections in Tortoises

Virus Detection. The detection of torchiviruses in tortoises has most often been described via isolation in cell culture. Virus can be isolated on TH-1 (*Terrapene* heart cells, ATCC CCL-50). An RT-PCR for the detection of viral RNA has also been developed.[184] The best samples for virus detection in live animals are oral swabs. In dead animals, samples from the entire gastrointestinal tract (tongue to cloaca) can all be used for virus detection. Virus is frequently also found in other tissues (including liver, kidney, heart, brain, and lung) (see Table 30.3).

Rafiviruses have not been isolated in cell culture. A PCR for their detection in tissues from affected animals has been described.[180] It has been successfully used to detect virus in liver and kidney samples from animals in the original disease outbreak.

Serology. Serological detection of antibodies against torchiviruses can be carried out using virus neutralization methods. Antibodies against this virus are frequently found in tortoises in Europe, and low titers have also been detected in wild-caught tortoises in Turkey.[139] It is unknown how closely related all torchivirus isolates are to one another, so cross-reactivity among these viruses and between these viruses and other picornaviruses is not yet understood.

CALICIVIRIDAE

Caliciviruses (CVs) infect a broad range of animals, and most individual CV species exhibit a natural host restriction. However, the vesicular exanthema of swine virus (VESV) is an exception, with a broad host range. Transmission is generally via contaminated food, water, fomites, and sometimes via aerosolization.[185] Caliciviruses can be inactivated using standard virucidal disinfectants (e.g., bleach and acid-based disinfectants) very quickly and are generally inactivated by alcohol formulations used for hand disinfection.

In reptiles, CVs closely related to the San Miguel sea lion CV have been isolated from four different snake species—Aruba island rattlesnakes, a rock rattlesnake (*Crotalus lepidus*), and an eyelash viper (*Bothriechis* [*Bothrops*] *schlegeli*)—as well as from Bell's horned frogs (*Ceratophrys ornata*) in a single collection in the United States.[186] Some, but not all, of the animals from which virus was isolated had enteritis and hepatitis. A transmission study with prairie rattlesnakes showed that these animals could be infected and the virus could be reisolated (in one out of two snakes inoculated), but no pathognomonic disease was observed in the infected snake. Transmission of the virus to pigs led to seroconversion but not disease.[186] A PCR for the detection of reptile calicivirus, as well as other vesiviruses, has been described,[187] but no information is available on the prevalence of this virus in reptiles.

FLAVIVIRIDAE

All of the flaviviruses described in reptiles belong to the genus *Flavivirus*, which contains viruses that are transmitted by hematophagous arthropods (arboviruses). Some flaviviruses are able to infect and replicate in a wide variety of vertebrate hosts (including mammals, birds, and reptiles). The focus of much interest in flaviviruses of reptiles has been the role of these animals as reservoirs for human disease.

Antibodies against various flaviviruses, including St. Louis encephalitis virus, Powassan virus, Japanese encephalitis virus, and West Nile virus (WNV), have been found in chelonians, snakes, and crocodilians in various parts of the world.[18,188] Japanese encephalitis virus has been isolated from Chinese rat snakes in Korea.[189] Transmission studies with Japanese encephalitis virus have shown that lizards can be infected with this virus both by parenteral inoculation and, in some species, by feeding on infected mosquitoes. Infected animals develop viremia, and the development of viremia is temperature dependent.[190] No clinical signs of disease were reported in the lizards. A flaviviruslike virus was isolated from a leopard tortoise with epistaxis, cloacal hemorrhage, biliverdinuria, and anemia. Herpes and adenoviruses were isolated from other animals in the same collection, and intraerythrocytic inclusions of unknown etiology were also described in these tortoises.[8,191] The most commonly described flavivirus in reptiles is West Nile virus.

WEST NILE VIRUS (WNV)

WNV is a flavivirus that primarily cycles between mosquitoes and birds. It is zoonotic, and care should be taken when dealing with suspected cases (especially in crocodilians). A number of transmission studies have been carried out with WNV and various reptile species. In one transmission study iguanas, snakes, and frogs became infected, and the iguanas and frogs both developed low-level viremias. No disease was reported in any of the infected animals.[192] Viremia was induced by subcutaneous inoculation into common garter snakes (*Thamnophis sirtalis*).[193] Another transmission study was able to induce a moderate viremia in four western fence lizards following inoculation with WNV.[194]

WNV has been shown to be pathogenic for crocodilians and causes high-titer viremias in these animals. Antibodies against WNV have been

detected in crocodilians in many different countries, including crocodiles at a commercial farm in Israel,[195] wild alligators in Florida,[196] and farmed and wild crocodiles in Mexico.[188,197] In the United States, WNV has been detected in disease outbreaks in American alligators in a number of different states, including Georgia,[198] Florida,[196] and Louisiana.[199] Infection of crocodilians can occur by bite from infected mosquitoes, orally by consumption of contaminated meat (e.g., infected horses), and by contact with viremic tankmates.[198,200] The development and duration of viremia are dependent on ambient temperature.[200] The highest mortalities have been found in young animals. Neurologic signs have included anorexia, tremors, swimming on their sides, spinning in the water, and opisthotonus. Oral lesions (stomatitis) have also been noted. Death may occur 24 to 48 hours after the appearance of clinical signs. The highest viral loads were detected in the livers of affected animals.[196,198] WNV has also been associated with lymphohistiocytic proliferative cutaneous lesions in American alligators. Round to ovoid lesions were described in the superficial dermis of infected alligators. No virus was detectable in the lesions, but animals with lesions consistently tested positive for antibodies against WNV and 97.5% tested positive for WNV RNA in pooled skin and liver-brain samples.[201] In a transmission study with alligators, viremia developed in all alligators infected by subcutaneous injection, with the time to development and duration of viremia dependent on temperature (32°C or 27°C), with longer duration of viremia at 27°C, while only tankmates of infected alligators kept at 32°C became infected. Viremia also developed after oral infection and also led to the infection of tankmates. Viral titers in serum of infected animals were sufficiently high to infect mosquitoes (*Culex quinquefasciatus*), and high virus loads were also documented in the cloaca.[200] WNV has been detected in mosquitoes associated with alligator farms.[202]

Identification of WNV Infections

Detection of WNV can be performed with the use of serum or whole blood in infected crocodilians. Viremic alligators have also been shown to shed virus via the cloaca.[200] In dead animals, virus can be detected in a number of tissues, including liver, lung, and blood. Liver has been shown to have the highest titers and yield the most positive results in American alligators (see Table 30.5).[196,198] Virus detection has been carried out by isolation in cell culture (e.g., Vero cells) or by detection of viral RNA using RT-PCR. A number of RT-PCR protocols have been described for the detection of WNV RNA in various animals. WNV is currently grouped into five different lineages, and the choice of RT-PCR will affect which lineages can be detected. Lineage 1 is found in North America, southern Europe, Asia, and Africa, whereas lineage 2 is found in southern Africa and has recently also been found in Europe. Real-time quantitative PCRs have been described for the combined detection of both lineages but have not been tested with crocodilian samples.[203]

Antibodies against WNV can be detected in tthe serum of infected crocodilians. In a transmission study with American alligators, antibodies were detected within 25 days of virus detection in infected animals. Methods used for the detection of anti-WNV antibodies have included a plaque reduction assay for the detection of neutralizing antibodies and an ELISA. The plaque reduction assay requires live virus and must therefore be carried out in a biosafety level 3 laboratory. An ELISA has been developed for the specific detection of antibodies against WNV in alligators.[204] Antibodies against WNV can cross-react with antibodies against other related flaviviruses.

TOGAVIRIDAE

The togaviruses detected in reptiles to date have all belonged to the genus *Alphavirus*, which contains viruses that have the ability to replicate in and be transmitted horizontally by mosquitoes. Most alphaviruses can infect a wide range of vertebrates, mostly birds and mammals, but several have also been reported in reptiles. Studies on alphaviruses in reptiles have mostly focused on the possible role of these animals for the transmission of alphaviruses to humans and livestock. This has led to a focus on persistence of alphaviruses in reptiles, particularly viral persistence over winter in temperate regions in the absence of mosquito activity.[205] Detection of alphavirus infection in reptiles has been carried out by detection of virus in blood or detection of antibodies. Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV), and antibodies against these viruses have been found in various chelonians, lizards, and snakes, including a number of wild-caught reptiles in the United States.[105] Antibodies against Venezuelan equine encephalitis virus (VEEV) have been found in free-ranging caimans in Brazil.[206] In a serosurvey of various frogs, turtles, lizards, and snakes in Alabama for antibodies against EEEV, seropositivity was detected most often in various snake species,[207] and repeated cycles of viremia were documented in garter snakes. EEEV RNA was detected in serum of wild-caught copperheads (*Agkistrodon contortrix*) and cottonmouths (*Agkistrodon piscivorus*) in Alabama.[208] Experimental transmission of WEEV and EEEV to garter snakes has shown that these animals can develop sufficiently high viremias to infect mosquitoes following hibernation.[18,209] Transmission studies with EEEV and WEEV in spotted turtles (*Clemmys guttata*) and Texas tortoises (*Gopherus berlandieri*) both led to viremia in injected animals.[210,211] Viremia was longer at lower temperatures than at high environmental temperatures. None of the transmission studies described any signs of clinical disease in infected reptiles.

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