EMERGING INFECTIOUS DISEASE OF REPTILES

La'Toya Latney, DVM, DEZCM (ZH), DABVP (Reptile & Amphibian)
Clinical Zoological & Exotic Animal Medicine
Exotic Companion Animal Medicine Service
University of Pennsylvania School of Veterinary Medicine, 3900 Delancey Street Office 3109
Philadelphia, PA 19104

Introduction
In practice, there are 3-4 staple infectious diseases among chelonians, lizards, and snakes present often. For bearded dragons (*Pogona vitticeps*)—there’s Oxyurids, *Isosphora*, and *Adenovirus*. For chelonians, there’s respiratory disease-causing triad of pathogens: *Mycoplasma*, *Herpes*, and *Ranavirus*. For snakes, *Cryptosporidium*, *Entamoeba invadens*, Inclusion Body Virus, and *Salmonella*-associated osteomyelitis. Among all orders, most clinicians rely on a bottle of ceftazidime to deal with suspected Gram-negative bacterial infections.

Some of these classic diseases still cause significant problems in captive reptiles, there are new disease, new treatment approaches and new classifications that may change how you treat. This review serves to revisit and compare the new and the old pathogens, provide updates on diagnoses, disinfection, potential treatment, and recommendations for improved biosecurity.

Fungal Update: *Chrysosporium*
We see unique wildlife populations fall victim to primary fungal pathogens, like *Geomyces destructans* or White Nose Syndrome in bats and *Batrachochytrium dendrobatidis* or Chytrid in amphibians. For the past 20 years, fungal infections have been responsible for the most severe population declines and extinctions ever witnessed, and 15% of reptile biodiversity is threatened by extinction (Fischer 2012). Fungal emergence is a worldwide threat to many species. Unfortunately, there are emerging primary fungal diseases that are following this trend with wild reptiles too, namely *Chrysosporium ophiodiicola* emergence in wild North American snakes (Allender 2011).

About the Pathogen: Formerly known has the *Chrysosporium* anamorph-Nanniziopsis vriessi (CANV) complex of organisms, reclassification based on molecular characterization have identified distinct genera, classified as *Nannizziopsis* or within the new genera *Paranannizziopsis* and *Ophidiomyces*. These organisms belong to the order Onygenales and reside in the same order as *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Microsporum*, and *Trichophyton* (Hoog 2000). They all act as primary contagious fungal pathogens. Fungal morphology among the genera is extremely similar, which makes identifying specific *Onygenales* species difficult. Therefore, DNA sequencing for is warranted for accurate pathogenic diagnosis.

Disease Hallmarks & Trade Names: Formerly known as *Chrysosporium* (CANV), *Nannizziopsis guarroi* causes severe ulcerative dermatomycosis, which can be extensive, deep, and become systemic and serves as the cause of “Yellow Fungus Disease” in bearded dragons (*Pogona vitticeps* and *P.barabata*) and has been documented in green iguanas. *Ophidiomyces oophidioicula* is the cause of the emerging disease known as “Snake Fungal Disease” in eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) and wild timber rattlesnakes (*Crotalus horridus*) (McBride 2012), with many studies revealing it as a strong contributor to wild snake mortalities in crotalids and water snakes.

Species Affected: Affected reptiles included crocodilians (Thomas et al., 2002), lizards (Paré et al., 1997; Martel et al., 2006; Bowman et al., 2007; Abarca et al., 2008, 2009; Han et al., 2010; Hellebuyck et al., 2010; Johnson et al., 2011; Toplon et al., 2012), snakes (Nichols et al., 1999; Bertelsen et al., 2005; Paré and Jacobson, 2007; Bicknese, 2009; Eatwell, 2010; McLelland et al., 2010), and tuataras (*Sphenodon punctatus*) (Masters et al., 2016), leaving the host range of fungi ill-defined (Pare 2016).

Diagnostics & Treatment: Fungal culture temperatures at 25 to 35°C are best to grow isolates. Fungal PCR and subsequent sequencing are necessary for speciation. For *N.guarroi*, clinical resolution was
achieved with the use of oral ketoconazole 20mg/kg PO in q24h in combination with topical chlorhexidine and terbinafine (Arbaca 2008). Treatment with any triazole antifungals requires caution, as hepatotoxicity can be common. Itraconazole has been used and can cause toxicity in bearded dragons at 5mg/kg PO q24h. Voriconazole at 10mg/kg PO q24h has been shown to be better tolerated in bearded dragons (Van Waeyenbergh 2010). Terbinafine nebulizations have been shown to achieve serum levels that would be effective against SFD in cottonmouths (Lindemann 2017). In experimental studies, voriconazole and Itraconazole have been fatal or ineffective in cottonmouths and timber rattlesnakes.

Biosecurity: No formal disinfection studies are available. Removal organic debris from cages and cleaning with 2% chlorhexidine are recommended at this time. Use gloves when handling affected reptiles. The zoonotic potential of Chrysosporium sp. is unknown, however CANV has been documented in immunocompromised individuals (Steninger 2005).

Parasite Update: Cryptosporidium spp.
Cryptosporidium belongs to a genus of coccidian parasites. They are of significant concern in squamates as they can cause severe gastrointestinal disease. They have been documented in at least 75 reptile species (O'Donoghue 1995), however that number is increasing. There are new reports of unique Cryptosporidium infections in captive lizards (Kik 2010, Richter 2011) and in testudines (Griffin 2010, Richter 2012).

Disease Hallmarks and Old Host Updates: There are 2 major classes that have been shown to infect squamata (lizards and snakes), C. varanii (formerly saurophilum) and C. serpentis. C. serpentis has a tropism for the gastric mucosa, causing a mid-body swelling, regurgitation and chronic wasting disease. C. varanii has a tropism for the intestinal mucosa and causes chronic wasting disease. Extra-intestinal infections are rare, however a cryptosporidium novel isolate has been reported to cause chronic cloacitis and cystitis in two green iguanas (Iguana iguana) (Kik 2011). C. parvum and C. muris are often recovered on gastric and fecal lavages in serpents from their prey, however they have not been shown to infect reptiles (Graczyk 1998).

New Host Alert: Within the past 15 years, there have been rare reports of unidentified Cryptosporidium species found in testudines. More recently, a novel species of Cryptosporidium was isolated and characterized as C. ducismarci, which has been found to infect and cause intestinal pathology in a testudine species (Pedraza-Díaz 2009). C. ducismarci and has also been found in a ball python Python regius.

Diagnostics: Gastric washes and fecal samples may demonstrate the acid-fast staining coccidian. As the recovery can be limited in these samples, PCR of gastric washes or feces is recommended. DNA sequencing is required for species identification as species-specific morphological diagnosis of Cryptosporidium can be difficult.

Treatment: There are no curative treatment regimens for Cryptosporidium infections in testudines or squamata. In one case report, Hermanni's tortoises (Testudo hermanni) 100mg/kg Paromomycin PO q24h for in tortoises 7 days, then 3 times weekly for 3 months, with supportive fluid therapy and an immunostimulant (Richter 2012). Fecals were PCR negative 2 weeks after treatment and resolved clinical signs, however recurrence was noted 8 months after treatment. One study evaluated the use of Paromomycin in bearded dragons (Grosset 2011). A co-infection with Isospora amphiboluri was noted in both the control and treatment groups. Treatments of 100mg/kg PO q24h for 7 days then 360mg/kg PO q48h for 10 days revealed an absence of intestinal cryptosporidium on histopathology, however gout, nephritis and a concurrent Isospora infection were noted in the treatment group and control groups. Cryptosporidium PCR and acid-fast testing were not performed during fecal screening. Hyperimmune-bovine serum has been used to reduce Cryptosporidium shedding in Savanna monitors (Varanus exanthematicus) (Graczyk 2000) and in captive snakes (Graczyk 1998).

Biosecurity: Cryptosporidium is highly contagious and extremely environmentally stable. Quarantine all affected reptiles. Disinfection can be achieved with steam-treatment, formalin (10%), glutaraldehyde (2.65%), and possibly 5% to 10% ammonia solution may be effective on clean, smooth, impermeable surfaces, but must be used with care to prevent toxicity to humans or animals in the vicinity (Gibson
Adenovirus infections are becoming more recognized in snake species (Garner 2008, Pees 2010) and testudines (Rivera 2009, Schumacher 2012). The adenovirus epidemic we observe in agamid lizards is now more widespread and diverse than we previously thought. See Table 1.

**Adenovirus Update**

Adenovirus infections began plaguing the North American and European captive bearded dragon population in the 1980s and is responsible for significant morbidity and mortality in the species (Jacobson 1986, Moorman 2009, Papp 2009). Clinical hallmarks may vary but includes weight loss, anorexia and diarrhea secondary to hepatic necrosis. CNS signs include circling, abrupt paresis, and opisthotonus. A severe vasculitis, hemorrhagic urates, and coelomic effusions may also be noted. Recent studies support that multiple genotypes of Agamid adenovirus 1 exist (Parker 2009). Often thought of as a disease problem in European and North American captive dragons, in 2011 the first report of adenovirus came from the animal’s native range, Australia (Hyndman 2011). Host fidelity among the lizard strains has also come into question. Most recently a helodermatid strain, HeAdV2, was reported in a captive bearded dragon in the United States (Wellehan 2012). A new adenovirus sequence was just sequenced from wild caught *Anolis* lizards in the United States, challenging the notion that this is just a disease seen in captive species (Asher 2013).

**New Host Alert:** Adenovirus has been identified as the cause of a virulent outbreak in Sulawesi tortoises (*Indotestudo forstenii*) that were confiscated following illegal importation (Rivera 2009). Clinical signs can vary, but include severe lethargy, anorexia, mucosal ulcerations, nasal and ocular discharge, and diarrhea. Sulawesi Adv1 has also been reported in a Burmese star tortoise (*Geochelone platynota*) that died without clinical signs and in an impressed tortoise (*Manouria impressa*). Both tortoises were housed at the same facility where the infected and confiscated Sulawesi tortoises resided (Schumacher 2012). Adenovirus has also been reported in an ornate box turtle (*Terrapene ornata ornata*) co-infected with *Mycoplasma agassizii* in Hungary (Farkas 2009). The ornate turtle virus’ recent genetic characterization may suggest a novel genus of adenovirus (Andor 2013).

**Diagnostics, Treatment and Biosecurity:** PCR or TEM of the feces is warranted. Treatment is supportive, including fluid therapy, warm water soaks, systemic and topical antibiotics, and nutritional support. Adenoviruses are very environmentally stable. Formal disinfection studies are lacking. Remove organic debris with soap and water followed by use of a 10% bleach solution on cages and hard surfaces, allowing for a 10-minute contact time (Slomka-McFarland 2006). Quarantine new dragons, snakes and tortoises upon entry into new collections and submit feces for PCR and/or TEM.

**Ranavirus Update**

Iridoviruses are large DNA viruses that are known to infect a diverse range of hosts, and have shown intraclass and cross-class host shifts. The current Iridoviridae family contains 5 genera; the genus *Ranavirus* infects fish, amphibians, reptiles and the genus *Iridovirus* infects insects and squamates (Jancovich 2010). Insect iridovirus strains have been recovered from lizard species (Weinmann 2007, Just 2001). Frog virus 3 (*Ranavirus*) is an OIE (Office International des Epizooties) reportable disease and is responsible for mass mortality in wild and captive amphibians populations. See Table 1.

**New Host Update:** Epidemiologic studies reveal FV3 is responsible for severe disease in wild eastern box turtles *Terrapene carolina carolina* (Johnson 2008) and experimental infections have been achieved in red-eared sliders *Trachemys scripta elegans* (Allender 2013). Ranavirus is responsible for significant morbidity and mortality in turtle and tortoise populations in the United States (Gibbons 2013). Clinical signs include severe conjunctivitis, palpebral edema, ocular and nasal discharge, oral plaques and abscessation, and severe subcutaneous edema of the neck.

**Treatment, Diagnostics:** Treatment is supportive, including fluid therapy, warm water soaks, systemic and topical antibiotics, nutritional support, Vitamin A and D₃ supplementation, and potential anti-viral therapy (Gibbons 2013). A diagnosis can be made based on highly suggestive clinical signs, and PCR of oral, cloacal and whole blood samples (Allender 2011).
Biosecurity: Quarantine sick testudines from other turtles and from amphibian collections. Chlorhexidine (0.75%), sodium hypochlorite (3.0%), and Virkon S (1.0%) inactivate ranaviruses after 1 minute of exposure. Potassium permanganate at 2 ppm or 5 ppm is not recommended because it was ineffective at inactivating the virus at 5 ppm (Bryan 2009).

Inclusion Body Virus Update
The causative agent of Lymphocytic choriomeningitis virus in rodents and the cause of callatrichid hepatitis, Arenavirus (King 2011), has now been identified as the causative agent of Inclusion Body Virus (IBD) in boid snakes (Stenglein 2012, Bodewes 2013).

Disease Hallmark: IBD is characterized as a highly contagious and fatal disease of captive boids. Pythons decline faster than boas from clinical signs, which include opisthotonus, loss of righting reflex, head tilt, disequilibrium, incoordination and sudden death. Some boa carriers may be asymptomatic and survive with infections for years.

Diagnostics, Biosecurity: The sensitivity of identifying intracytoplasmic viral inclusions in circulating white blood cells (lymphocytes and heterophils) has not been formally studied but has been clinically reported (Chang 2010, Pees 2010). Hepatic or esophageal tonsil biopsies are recommended to confirm disease pending the development of new molecular screening tools. Chang and colleagues are validating a specific blood-based immunodiagnostic test for potential use as a screening tool. Quarantine all new boids from established collections is strongly recommended pending biopsy confirmation or CBC confirmation of health status. Snake mites, *Ophionyssus natricis*, are thought to serve as vectors and have been associated with IBD outbreaks. It is imperative to treat and limit infestations promptly (Chang 2010).

Bacteria Update: *Devriesea agamarum*
There is a newly discovered species of Gram-positive bacteria, *Devriesea agamarum*, which causes significant dermatological disease in *Uromastyx* sp. and agamid lizards (Martel 2008). Thankfully, several studies provide effective treatment and disinfection regimens to eliminate this pathogen from collections.

Disease Hallmark: Severe hyperkeratotic dermatitis, cellulitis, and potential septicemia

Diagnosis & Treatment: *D. agamarum* is a short (1-2mm), non-spore forming, nonmotile, non-acid fast Gram-positive rod. Debridement and intramuscular administration of cefiofur at 5mg/kg q24h for 18 days in bearded dragons and 12 days in *Uromastyx* sp. resulted in clinical resolution. Enrofloxacin was not effective (Hellebuyck 2009).

Biosecurity: *D. agamarum* can exist in humid sand and distilled water for over 5 months, and remains in dermal crusts for up to 57 days. Hard surfaces can be disinfected with the following solutions allowing for a 5-minute contact time minimum: sodium hypochlorite (0.05%–0.5%), chlorhexidine (0.05%–0.5%), boric acid (0.01%), and ethanol (70%) (Hellebuyck 2011).

Conclusions
Table 1 provides a quick glance at some of the emergent diseases in captive and wild reptiles. Host ranges are less specified than what we previously assumed for a number of infectious disease agents. Complete reviews can be found in the following articles; "Selected Emerging Infectious Diseases of Squamata" (Latney, Wellehan 2013) and “Emerging Infectious Diseases of Chelonians” (Gibbons, Steffes), published in *Veterinary Clinics of North America: Exotic animal practice* 16.2 (2013).

Table 1: Summary of Emerging Infectious Diseases in Reptiles

<table>
<thead>
<tr>
<th></th>
<th>Testudines</th>
<th>Saurians</th>
<th>Ophidians</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td>Adenovirus</td>
<td>Siadenovirus (Sulawesia Adv1) and a new partially characterized genus</td>
<td>AgAdv1, AgAdv2, EuAdV1, GeAdV-1, ScAdV-1, ChAdv-1, HeAdV1, HeAdV2, and Varanid AdV1, Anolis Adv1</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td>--------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Iridovirus</td>
<td>Frog Virus 3 (FV3)</td>
<td>FV3, Invertebrate Iridovirus</td>
<td></td>
</tr>
<tr>
<td>Arenavirus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Parasites   | Cryptosporidium | C. ducismarci, & a separate novel Genus- partially characterized | C. serpentis, C. varanii, C. baileyi, several unidentified strains | C. serpentis, C. varanii, C. baileyi, several unidentified strains |

| Fungal Disease | Chrysosporium | Chrysosporium sp. in Carettochelys insculpta | CANV, CANV-associated sp, unidentified species, C. guarro | C. ophiodiicola |

**References:**  
A full reference list is available upon request, email llatney@vet.upenn.edu