Way, K., Haenen, O., Stone, D., Adamek, M., Bergmann, S. M., Bigarré, L., ... & Waltzek, T. (2017). Emergence of carp edema virus (CEV) and its significance to European common carp and koi Cyprinus carpio. *Diseases of aquatic organisms*, *126*(2), 155-166.

**Abstract:** Carp edema virus disease (CEVD), also known as koi sleepy disease, is caused by a poxvirus associated with outbreaks of clinical disease in koi and common carp Cyprinus carpio. Originally characterised in Japan in the 1970s, international trade in koi has led to the spread of CEV, although the first recognised outbreak of the disease outside of Japan was not reported until 1996 in the USA. In Europe, the disease was first recognised in 2009 and, as detection and diagnosis have improved, more EU member states have reported CEV associated with disease outbreaks. Although the structure of the CEV genome is not yet elucidated, molecular epidemiology studies have suggested distinct geographical populations of CEV infecting both koi and common carp. **Detection and identification of cases of CEVD in common carp were unreliable using the original PCR primers. New primers for conventional and quantitative PCR (qPCR) have been designed that improve detection, and their sequences are provided in this paper.** The qPCR primers have successfully detected CEV DNA in archive material from investigations of unexplained carp mortalities conducted >15 yr ago. Improvement in disease management and control is possible, and the principles of biosecurity, good health management and disease surveillance, applied to koi herpesvirus disease, can be equally applied to CEVD. However, further research studies are needed to fill the knowledge gaps in the disease pathogenesis and epidemiology that, currently, prevent an accurate assessment of the likely impact of CEVD on European koi and common carp aquaculture and on wild carp stocks.

**Etiology:** Carp edema virus disease (CEVD) = koi sleepy disease (KSD)

* A poxvirus, causes morbidity and mortality in koi and carp
* Optimum temperature = 15-25 C
* Japan in 1970s → USA in 1990s → Europe, India, China, Brazil in 2000s

**Clinical Signs:**

* Lethargic and lying motionless at the bottom of the pond
* Able to swim for short distances when around but return to motionless state
* Mortality up to 75-100% in juvenile koi
  + At optimal temps, acute mortality within 2-3 days
  + At low temps, extended disease course with less mortality
* External lesions common in juveniles:
  + Erosions
  + Skin hemorrhage
  + Edema of underlying tissue
* External lesions common in adults:
  + Enophthalmia
  + Anorexia
  + Ulcers around mouth and base of fins
  + Pale and swollen gills
  + Inflammation around anal vent
* Secondary bacterial infections are common

**Diagnosis:**

* Gill = best tissue for testing
* Newer PCR and qPCR is most commonly used
* Light and transmission electron microscopy can be used but may be insensitive to detect virus
* Virus isolation has not been successful

**Management:**

* No treatment
* Prevent disease by sourcing from reliable breeders having good biosecurity (e.g. quarantine)
  + Reduce stress on new fish by avoiding overstocking, ensuring good water quality
  + Don’t harvest fish during optimal temperatures
* In Japan, CEVD is managed with 0.5% saline water after stressful events to reduce toxicity and nitrite (and therefore reduce overall stress of the fish)
* Amur wild carp were resistant to both CEV genotype I and II
* Asymptomatic carriers have not been described

**Conclusions:** Carp edema virus is a poxvirus that causes lethargy, skin lesions, and death in koi and carp. Accurate diagnosis and prevention is key.

Morgan, C. N., López-Perez, A. M., Martínez-Duque, P., Jackson, F. R., Suzán, G., & Gallardo-Romero, N. F. (2019). Prevalence of antibodies to orthopoxvirus in wild carnivores of northwestern Chihuahua, México. *Journal of wildlife diseases*, *55*(3), 637-644.

Abstract: The distribution of orthopoxviruses (OPXVs) across the North American continent is suggested to be widespread in a wide range of mammalian hosts on the basis of serosurveillance studies. To address the question of whether carnivores in northwestern Mexico are exposed to naturally circulating OPXVs, wild carnivores were collected by live trapping within four different habitat types during fall of 2013 and spring of 2014 within the Janos Biosphere Reserve in northwestern Chihuahua, Mexico. A total of 51 blood samples was collected for testing. Anti-OPXV immunoglobulin G enzymelinked immunosorbent assay, western blot, and rapid fluorescent focus inhibition test (RFFIT) assays were conducted. **About 47% (24/51) of the carnivores tested were seropositive for anti-OPXV binding antibodies and had presence of immunodominant bands indicative of OPXV infection. All samples tested were negative for rabies virus neutralizing antibodies by RFFIT, suggesting that the OPXV antibodies were due to circulating OPXV, and not from exposure to oral rabies vaccine (vacciniavectored rabies glycoprotein vaccine) bait distributed along the US–Mexico border.** Our results indicated that there may be one or more endemic OPXV circulating within six species of carnivores in northwestern Mexico.

* Background:
  + Orthopoxviruses – monkeypox (zoonotic), vaccinia virus (economic losses from livestock), cowpox virus (pets, zoo animals).
    - Worldwide distribution, naturally endemic on all continents except Aus and Antarctica.
    - Three species endemic to NA: Skunkpox, raccoonpox, volepox.
    - Natural reservoir hosts of NA OPXVs remain unknown (probably rodents). Ab detected in many mammals.
    - Predators take a disproportionate number of prey diseased and infected with parasites. Prey that are sick are more vulnerable to predation.­­­­­
    - Presence of antirabies or anti-OPXV antibodies in carnivores of northerwestern Mexico could be indicative of an exposure to oral rabies vaccine or exposure to natural endemic OPXV.
* MM:
  + Blood from wild carnivores from five areas during Oct, Nov 2013 and Apr, May 2014.
  + Trapped, sedated, collected blood. Serologic testing for OPXV (ELISA).
  + True positive – Positive ELISA + WB testing.
* Results:
  + WB – 59% showed bands consistent with bands commonly found in OPXV infections.
  + ELISA – 51% anti-OPXV antibodies.
  + 47.1% animals suspected previous exposure or current infection based on definition of true positive. Raccoons highest seroprevalence (75%), coyotes 67%, kit foxes 55%.
    - 60% grassland ecotone, 59% grassland, 36% shrubland, 20% forest.
* Discussion:
  + Predator-prey-pathogen interactions may play a role in natural disease transmission linkages of OPXV in NA.
  + All Blood samples were also tested for presence of rabies Ab and were negative, so the OPXV positives in this study are not from exposure to ORV baits.
  + This study supports prev studies that raccoons harbor highest seroprevalence rates in NW Mexico. New evidence for coyote, gray fox, kit fox, Am badger in this region.
* Takeaway: OPXV fairly widespread across carnivore groups in NW Mexico, raccoons highest prevalence in general, predator-prey-pathogen interactions important (rodent reservoir).

Journal of Zoo and Wildlife Medicine 50(4): 803–812, 2019

**WHOLE GENOME SEQUENCING OF AN AVIPOXVIRUS ASSOCIATED WITH INFECTIONS IN A GROUP OF AVIARY- HOUSED SNOW BUNTINGS (*PLECTROPHENAX NIVALIS*)**

Rozenn Le Net 1, Chantale Provost 2, Christian Lalonde 3, Lyette Régimbald 4, Francois Vézina 4, Carl A Gagnon 5, Stéphane Lair 6

**ABSTRACT:** Avipoxvirus infections have been reported in both free-ranging and domestic birds worldwide. **Fowlpox and canarypox viruses belong to the genus *Avipoxvirus* among the virus family Poxviridae**. They cause cutaneous lesions with proliferative growths on the unfeathered parts of the skin and/or diphtheritic lesions generally associated with necrosis in the upper respiratory and digestive tracts. **In this study, a poxvirus has been identified in wild-caught snow buntings (*Plectrophenax nivalis*) housed in an outdoor aviary in the region of Rimouski, Quebec**. **During the falls and winters of 2015 and 2016, eight snow buntings affected by this infection were examined.** Macroscopic and microscopic lesions observed were characteristic of an avipoxvirus infection. Electron microscopy imaging of an ultrathin section of the histopathological lesions of two birds confirmed the presence of the poxvirus. Afterward, the presence of the poxvirus was confirmed in three birds by a specific polymerase chain reaction assay that amplified a segment of the gene encoding the fowlpox virus 4b core protein. A 576-nucleotide amplicon was obtained from one of them and sequenced. **The analyses revealed a 99% homology to other previously described avipoxviruses**. Using high-throughput sequencing, almost the entire viral genome of this avipoxvirus was revealed and found to possess a 359,853-nucleotide sequence in length. **Bioinformatic analyses revealed that the virus was genetically related to canarypox virus**. To our knowledge, this is the first confirmed case and full description of a poxviral infection in this species. **This episode suggests a high susceptibility of this northern species of passerine to avipoxviruses circulating in southeastern Canada during the summer months. Even if the source of the viral infections remains undetermined, transmission by local biological vectors is suspected. Management of poxviral infections in snow buntings housed outdoors in southeastern Canada could rely on the control of biting insects.**

**Study Design**: Case series with histopathology and advance molecular diagnostic workup

**Goal:**

* Report the clinical presentation, pathological findings, genomic sequence, and phylogenetic classification of an avipoxvirus associated with mortalities in snow buntings

**Key Points:**

* Avipoxviruses belong to the Poxviridae family in the Chordopoxvirinae subfamily and are made up of three major clades:
  + Clade A, of which fowlpox virus is the most well-known species
  + Clade B, of which canarypox virus is the most well-known species
  + Clade C, which is made up mostly of psittacinepox virus
* Sporadic cases of poxviral infections, characterized mainly by wart-like masses on unfeathered areas have been reported in various free-ranging birds
  + Epizootics have also been reported in colonial nestling birds, such as lesser flamingos, southern giant petrel, and Laysan albatross
* Reports of poxvirus infections in birds under human care are not as common as in free-ranging birds
  + Poxviral infections have been documented in zoo housed flamingos as well as in a captive breeding program of houbara bustards (*Chlamydotis spp.*)
  + This lower occurrence is likely due to a lower exposure to blood-sucking or biting arthropods, which are believed to be the main route of transmission
* During the falls and winters of 2015 and 2016, outbreaks of poxviral infections have occurred in snow buntings housed outdoors at the University of Quebec at Rimouski
  + All affected birds presented clinical signs characterized by the presence of a single or multiple small dermal, usually proliferative lesions on the legs, wings, and face
  + Several treatments were attempted without success, including diluted chlorhexidine bath, anti-inflammatory topical ointment, Derma Gelt, topical treatment with a dexamethasone and tobramycin ophthalmic ointment, and oral tetracycline/neomycin
  + One of the affected birds (#8) had been vaccinated against fowlpox virus and avian encephalomyelitis six months prior to the onset of the clinical signs
  + None of the birds from the other species kept in this facility exhibited lesions suggestive of an avipoxvirus infection
* Every bird displayed proliferative cutaneous lesions characterized by a thickening of the skin or oral mucosa forming occasionally ulcerated masses covered with crust
  + These masses were located on toes, intertarsal articulations, eyelids, wrists, elbows, and oral mucosa
* Histopathologic examination of the cutaneous lesions showed lesions characteristic of avipoxvirus infection including:
  + The presence of numerous large eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies) filling a large proportion of the cytoplasm of epidermal cells
* Even though poxvirus was confirmed in only 4/8 cases, a diagnosis of poxviral infection was given to all eight birds due to the presence of highly characteristic histologic lesions
* Mortality rate with the cutaneous form of poxvirus infection is usually low
  + However, in the epornitic described here, all the birds that showed pox lesions either died naturally or were euthanized
* TEM pictures clearly demonstrate the presence of poxvirus in two samples
  + However, discordance in the PCR results in one of the positive samples obtained by TEM was observed
  + This discrepancy could be explained by the difference in homology of the primers used
* Moreover, a divergence between negative PCR results and positive characteristic histologic lesions was also observed
  + DNA extracted from deparaffinized tissue often leads to a lower DNA quality and yield, and half of the samples tested by PCR were from deparaffinized tissues
  + Thus, the inconsistency might come from the type of samples analyzed by PCR in combination with an 80% homology of one of the primers used, which could overall reduce PCR sensitivity
* Once sequenced, it appeared that the genome length and organization of the snow bunting avipoxvirus was very similar to that of canarypox viruses, therefore belonging to avipoxvirus clade B
* Little is known about diseases and causes of death in snow buntings
  + The lack of knowledge in diseases of snow buntings may stem from their small size, their rural habitat with low human population, and their northern distribution range
* The occurrence of this episode in a captive setting within an unusual summer range for this species suggests that captivity created artificial conditions leading to a poxviral infection
  + Because the birds were kept in southern Quebec during the warm months of the year, they were exposed to unusual vectors, including blood-sucking or biting arthropods, that could have facilitated transmission of a virus to which this species is naïve
  + Seven of the eight cases were documented in the fall
    - This seasonal pattern, following the summer season with a high abundance of mosquitos, is similar to what is observed in other bird species and supports the hypothesis of an arthropod-borne transmission
* The captive setting might have enhanced the transmission of the avipoxvirus by increasing bird-to-bird contact due to their confinement
  + As for most viral diseases, transmission of avipoxviruses is promoted when the host density increases
  + Alternatively, poxvirus could be enzootic in snow buntings, and clinical cases could have occurred due to the stress associated with the captivity
* No treatment attempted on symptomatic birds proved to be successful in the described cases
  + No specific treatment of avipoxvirus infection exists
  + Management of an avipoxvirus epornitic in a captive collection should rely mainly on prophylaxis as well as isolation of symptomatic birds

**TLDR:**

* A poxvirus was identified in wild-caught snow buntings (*Plectrophenax nivalis*) housed in an outdoor aviary in the region of Rimouski, Quebec
* Bioinformatic analyses revealed that the virus was genetically related to canarypox virus
* Give the observed 100% mortality, northern species of passerine may be highly susceptible to avipoxviruses circulating in southeastern Canada during the summer months
  + Transmission by local biological vectors is suspected

**Related Articles**

*No references fall within the ACZM reading list (2017-Present)*

Novel poxviral infection in three finch species illegally imported into Trinidad, West Indies, with implications for native birds.

Suepaul RB, Seetahal JF, Oura C, Gyan L, Ramoutar VV, Ramkissoon V, Sahadeo N, Carrington CV.

Journal of Zoo and Wildlife Medicine. 2019;50(1):231-237.

Abstract: *Oryzoborus angolensis (Lesser Seed-Finch), Oryzoborus crassirostris* (Large-billed Seed-Finch), and *Sporophila intermedia* (Grey Seedeater) are finch species native to the Caribbean island of Trinidad. These species are locally trapped and kept for their song, but with declining native populations, enthusiasts have turned to illegally importing birds from the South American mainland. **The smuggling of wild birds from South America poses significant disease risks to the native bird species of Trinidad. Herein we describe the first case of poxviral infection in these illegally imported birds in Trinidad and partial genome sequence of the causative agent.** Phylogenetic analysis of the 4b core protein sequence indicated that the avian poxvirus identified was most closely related to a 2012 avian pox sequence from Brazil, with 96.2% and 98.1% identity at the nucleotide and amino acid levels.

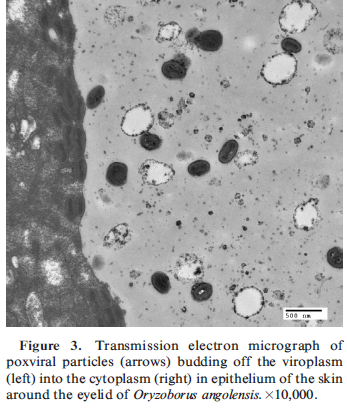
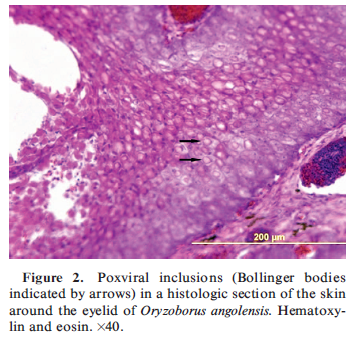
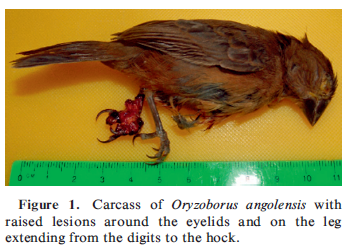
**Background**:

* Avian poxviruses: Genus *Avipoxvirus*; family *Poxviridae*
  + Dry/cutaneous form: 1-5 mm nodules on unfeathered areas
  + Wet/diptheritic form: necrotic caseous membrane in upper resp tract, mouth, pharynx
  + Viremic form: described, high mortality.
* 10 spp of poxvirus currently recognized: *Fowlpox, Canarypox, Juncopox, Mynahpox, Psittacinepox, Sparrowpox, Starlingpox, Pigeonpox, Turkeypox, Quailpox virus*
  + Three main clades: A (fowlpox), B (canarypox), C (Psittacinepox)

**Key points**:

* Lesser Seed-Finch*,* Large-billed Seed-Finch, and Grey Seedeater imported from South America (suspect Venezuela) into Trinidad presented with suspected pox lesions.
* Confirmed with histo, electron microscopy of viral particles, and PCR for avipoxvirus
  + Histo: Bollinger bodies (large round eosinophilic cytoplasmic inclusions), epithelial hyperplasia and ballooning degeneration, inflammation
  + TEM: brick-shaped enveloped with a nucleocapsid, budding of inclusion membrane
  + PCR and sequencing: Canarypox-like virus, distinct but closest to Brazilian penguinpox
* 8/10 were juvenile, consistent with expectation of more severe disease in juveniles
* Mosquito transmission is a likely factor
* Virus may be shed for up to 13 mo after clinical disease

**Conclusion**: Illegal importation of finches into Trinidad contributes to decline of native finch spp. (phenotypically distinct) by introduction of poxvirus and displacement of the native birds.



"Outbreak and treatment of carp edema virus in koi (Cyprinus carpio) from northern California." *Journal of zoo and wildlife medicine* 49.3 (2018): 755-764.

Abstract: **Carp edema virus (CEV) is the causative agent of carp edema virus disease (CEVD),** also referred to as **koi sleepy disease**, which is an **emerging disease of global concern** that may cause **high rates of morbidity and mortality** in common carp and ornamental koi (Cyprinus carpio). This article reports the third confirmed outbreak of CEVD in California. In June 2015, three koi presented with clinical signs of **cutaneous lesions**, **severe lethargy, and** **signs of hypoxia**. All fish tested **positive for CEV by polymerase chain reaction (PCR)**. Euthanasia and complete necropsy were performed on two fish. The most significant necropsy findings included **necrotizing branchitis with marked interstitial edema, multifocal cutaneous ulcerations, and severe cutaneous edema. Treatment of the pond with 0.3–0.5% salt was recommended to the owner.** Approximately 7 wk later, a recheck visit was made to the pond. No mortalities had been noted since the initiation of the salt treatment. Physical examination revealed **a vast improvement but not complete elimination of the clinical signs of hypoxia and intermittent lethargy in the affected fish**. Gill biopsy samples from the two most affected fish were tested and remained PCR positive for CEV. **Subsequent recheck visits over 11 mo postdiagnosis and initiation of treatment showed continued improvement in most fish. Gill samples from all fish in the pond (n 1⁄4 9) were repeatedly tested by quantitative PCR for CEV, and all samples were negative**. This case series further confirms the global spread of CEV and the need for practitioners to be vigilant for outbreaks of this disease. If CEVD is suspected, **treatment with 0.3–0.5% salt** can be recommended to potentially mitigate the effects of this disease. However, **fish may remain potential carriers** of this pathogen, and strict biosecurity measures should continue to be enforced for any pond that has had a confirmed CEV outbreak.

Case Summary

* June 2015, three koi presented with clinical signs of cutaneous lesions, severe lethargy, and signs of hypoxia
* All fish tested positive for CEV by polymerase chain reaction (PCR).
* Necropsy findings included necrotizing branchitis with marked interstitial edema, multifocal cutaneous ulcerations, and severe cutaneous edema.
* Treatment of the pond with 0.3–0.5% salt was recommended to the owner.
* Physical examination revealed a vast improvement but not complete elimination of the clinical signs of hypoxia and intermittent lethargy in the affected fish.
* Subsequent recheck visits over 11 mo postdiagnosis and initiation of treatment showed continued improvement in most fish.
* Gill samples from all fish in the pond were repeatedly tested by quantitative PCR for CEV, and all samples were negative

Discussion

* Characteristic CS = lethargic, “sleepy” behavior, lying at the bottom of the pond in 15-25C temperatures
* Originally just Japan, by 2013 in nine countries including US
* Unknown if fish clear viral infection after exposure or if become asymptomatic carriers (evidence suggests the latter)
* CEV is possibly immunosuppressive based on frequent finding of concurrent infections (in this case CyHV-1)
* Periocular inflammation and erythema seen in one fish has not been reported in CEV before

Take home: third PCR-confirmed outbreak of CEV in California, confirms global spread of CEV. Salt treatment can be recommended but fish may still be potential carriers