

TESTUDINID HERPESVIRUSES

Animal Group(s) Affected	Transmission	Clinical Signs	Severity	Treatment	Prevention and Control	Zoonotic
All tortoises are considered susceptible.	<p>Experimental work has shown that intranasal and intramuscular inoculation is followed by development of the disease.</p> <p>Close contact is considered one of the most relevant events for natural transmission to occur.</p> <p>It is not clear if aerosolization plays a significant role.</p>	Classic, but not necessarily always occurring, clinical signs include diphtheronecrotic stomatitis and glossitis, nasal, ocular and oral discharge, conjunctivitis, lethargy, anorexia, respiratory and neurological signs.	<p>Depends of the species affected and on the viral genotype involved. TeHV-1, -2, and -3 have been associated with clinical disease, while TeHV-4 has not.</p> <p>Close to 100% morbidity and mortality have been reported for naïve Hermann's tortoises while Greek tortoises are considered more resistant.</p>	Antivirals (e.g., acyclovir and gancyclovir) have been shown to be effective <i>in vitro</i> .	Separation of the diseased individuals from those clinically healthy animals; serologic and molecular testing of the exposed individuals; six months quarantine and serological testing; avoiding species mixing and crowding; disinfection of the enclosures with virucidal agents.	No

Fact Sheet compiled by: Francesco C. Origgi

Sheet updated on: 30 August 2013

Fact Sheet Reviewed by: David E. Hannon; Douglas R. Mader

Susceptible animal groups: All tortoises are considered susceptible.

Causative organism: Testudinid herpesviruses (TeHVs) (previously known as tortoise herpesviruses) have four currently known genotypes: TeHV-1, -2, -3 and -4. In diseased individuals, TeHV-1, -2 and -3 have been detected or isolated while TeHV-4 has been detected in a single clinically healthy Bowsprit tortoise (*Chersina angulata*).

Zoonotic potential: No

Distribution: TeHV-1 and -3 have been detected worldwide. TeHV-2 has been detected only in the US until now. TeHV-4 has been detected in the US in a clinically healthy, imported Bowsprit tortoise. TeHV-1 and -3 have been detected more commonly in the genus *Testudo* but also in several other tortoise species not autochthonous of the Eurasian area. TeHV-2 has been detected only in desert tortoises (*Gopherus agassizii*) up to date. It is likely that at least for some of the genotypes, the current area of distribution might only partially overlaps with their original ones because of the extensive movements that the species have undergone especially because of the pet trade. According to multiple studies conducted on wild tortoises, the following geographical classification of the genotypes has been proposed: TeHV-1 and -3, Eurasian; TeHV-2, American; TeHV-4, African.

Incubation period: Following experimental infection in Greek tortoises, the first clinical signs were

TESTUDINID HERPESVIRUSES

recorded 7 to 12 days post inoculation. The overall course of the disease lasted 2.5 weeks.

Clinical signs: The disease originally was called “stomatitis and rhinitis disease” or “running nose” and it was named after its most common clinical signs that include a diphtheronecrotic stomatitis and a glossitis with nasal, oral and eye discharge. The discharges are generally clear, intermittent and recurrent. Monolateral or bilateral recurrent conjunctivitis is also relatively common. Cervical edema has also been reported, as has epistaxis in severe cases. The oral plaques have been shown to develop and eventually fully regress in the survival animals within a 2.5 weeks period of time during an experimental transmission study in Greek tortoises. Following the loss of the crusts covering the healing plaques, no scar can be detected after the resolution of the oral lesions. A band of chalky white material can be observed along the rim of the external aspect of the mandible after the oral discharge has resolved. Respiratory and neurological signs might follow along more general signs such as lethargy and anorexia. Not all the clinical signs described above can be detected in the same diseased individuals and some infected tortoise might not develop obvious clinical signs at all.

It is very important to consider that none of the clinical signs described above is specific for TeHVs, since similar oral plaques have also been described in tortoises infected with iridovirus and virus X (picornavirus), although these latter cases represent far less common occurrences than TeHVs infections. Additionally, nasal discharge and conjunctivitis, in absence of stomatitis, are commonly described in tortoises infected with *Mycoplasma agassizii* (an etiologic agent of the upper respiratory tract disease-URTD).

Post mortem, gross, or histologic findings: Classic gross lesions include diphtheronecrotic plaques over the mucosa of the oral cavity and the tongue, occasionally extending over the mucosa of the esophagus and of the trachea up to the lung. Hepatomegaly and enteritis have also been described. The histologic hallmark of the disease is the presence of eosinophilic to amphophilic intranuclear inclusions in most of the epithelial tissues. Inclusions can also be observed within the central nervous system either associated or not with inflammation. Inclusions are likely to be detectable for a limited time during the initial phase of the disease.

Diagnosis: The clinical diagnosis relies on the detection of the clinical signs described above. The clinical diagnosis requires the confirmation by laboratory testing. Multiple molecular and serological tests are available for the diagnosis of Testudinid herpesvirus infection.

Serology: The available serological tests comprise an ELISA and a serum neutralization test (SNT), while the molecular diagnostic tests comprise multiple PCR protocols for the partial amplification of different herpesvirus genes. The ELISA test has been developed to detect TeHV-3 exposure and it has been validated for Greek and Hermann’s tortoises. The test detects the large majority of the antibody developed by the host against the virus after seroconversion. Although the test can detect the exposure to TeHV-3 relatively early following the infection, for a reliable diagnosis it is recommended to test a suspected individual two times no less than 8 weeks apart. A modified version of the same test has been shown to be able to detect TeHV-2 exposure in desert tortoises, although in this format the test has not been fully validated. SNT similarly to the ELISA, allows the detection of the exposure of an individual to a Testudinid herpesvirus due to the presence of circulating anti-TeHVs antibody (serum neutralizing in this case) following seroconversion. This test can be applied to any species of tortoise and it requires live virus to be carried out. SNT can detect seroconversion to TeHVs 2-5 weeks after the ELISA test. For this reason, it is suggested to test the suspected animals two times 10-12 weeks apart for a reliable detection of the occurred seroconversion when using the SNT. Both ELISA and SNT show comparable specificity and sensitivity.

Molecular diagnosis: Several PCR protocols have been developed for the diagnosis of TeHVs infection. A PCR test targeting the partial sequence of the viral DNA polymerase gene is available for the specific detection of TeHV-1. Another protocol has been developed for the detection of the partial sequence of the helicase gene of TeHV-3. The same protocol allows also the detection of the homologous gene of the TeHV-1 genotype following a specific modification of the test conditions. A PCR test directed to the amplification

TESTUDINID HERPESVIRUSES

of the partial sequence of the ribonucleotide reductase (RR) (large subunit) of TeHV-3 has also been developed. This test can also amplify the homologous gene portion of TeHV-2, although a specific PCR protocol is also available for the partial amplification of TeHV-2 RR. Finally a PCR protocol, not specifically developed for TeHVs but more in general for the detection of the members of the family Herpesviridae is also available and allows the detection of all the 4 genotypes of TeHVs known up to date (partial amplification of the DNA polymerase gene; the target is a different region from that used for TeHV-1 amplification).

Histopathology: routine histopathologic diagnosis of TeHVs infection is more often considered a post-mortem diagnostic method. It is based on the detection of the classic intranuclear inclusions. An immunohistochemistry and an *in situ* hybridization method are also available for the detection of TeHVs antigen and DNA in tissue, respectively and can be used to enhance the sensitivity of the histopathological diagnosis. Electron microscopy is also commonly used to detect the presence of the virus in tissues.

Viral isolation: Viral isolation of TeHV-1 and -3 can be performed on reptilian cell cultures (TH-1 cells, subline B1, ATCC CCL-50). Classic cytopathic effects include cell rounding with cell detachment and lysis (plaque formation).

Material required for laboratory analysis:

Serology: 0.2-0.5 ml serum in plastic tube. Store at 4°C and ship refrigerated.

Virology: Pharyngeal swabs are collected for live animals. For dead animals, it is helpful to send the entire carcass if available or 1 g portions of each organ, ideally. If this approach is not possible, the head (including the tongue) of the suspected individual can be submitted. Place samples in viral transport media with antibiotic (1-2 ml for swabs and 3 ml per each tissue sample). Store the samples at 4°C for very short-term storage and -80°C for long-term storage. Ship samples refrigerated or on dry ice accordingly. Preserve the entire carcass and the head at 4°C and ship refrigerated immediately.

Molecular diagnostic: Same samples described for virology but in this case viral transport media is not required. Samples can be stored and shipped also as described for the virology samples, with the exception that for molecular diagnostic the samples can be stored also at -20°C if -80°C freezers are not available. If only formalin-fixed, paraffin-embedded tissues are available, whole tissue block(s) can be used. If not possible, please send 3 sections 20µ-thick each (per tissue block) in a plastic tube (DNase- and RNase-free). The samples do not need to be refrigerated.

Histopathology: The entire carcass is preferred if the carcass can be stored at 4°C and shipped immediately refrigerated. If the necropsy is performed *in situ* please collect routine samples of all the organs including the brain. Samples need to be placed into a container with 10% buffered formalin.

Relevant diagnostic laboratories:

From US, permits could be required for shipment to international laboratories:

Wildlife Diagnostic Laboratory at the Centre for Fish and Wildlife Health (FIWI)
University of Bern, Vetsuisse Faculty
Länggassstrasse 122
3012, Bern, Switzerland
+41 31 631 2443
Fax +41 31 631 2635
Francesco.origgi@vetsuisse.unibe.ch

Institut Für Umwelt- und Tierhygiene
Universität Hohenheim
Garbenstrasse 30

TESTUDINID HERPESVIRUSES

70599 Stuttgart, Germany
+49 711 459 22468
Fax +49 711 459 22431
Rachel.marschang@googlemail.com

Staatliches Veterinäruntersuchungamt
Westernfeldtrasse 1
32758 Detmold, Germany
+49 05231 911640
Contact person: Silvia Blahak

Veterinary Laboratory Agency
Weybridge, Woodham Lane, New Haw
Addlestone. Surrey. KT15 3NB
United Kingdom
Contact person: Sally Drury

College of Veterinary Medicine
University of Florida
2015 SW 16Ave.
32610 Gainesville, Fl, USA
352 294 4420
childressa@ufl.edu

Treatment: Acyclovir and gancyclovir have been shown to be effective against TeHV-3 *in vitro*. Acyclovir also has been used to treat infected animals at 80mg/kg PO SID or TID. An *in vivo* study in marginated tortoises (*Testudo marginata*) showed that a single administration of this dose acyclovir results in a serum concentration of the drug which is lower than that reported to be effective against the virus *in vitro*. Broad-spectrum antibiotics and supportive rehydration therapy have also been described as part as the therapeutic protocol proposed for TeHVs infected tortoises. The duration of the treatment may vary, but it should be no less than two to three weeks unless otherwise suggested by the clinical evaluation and laboratory testing.

Prevention and control: Tortoises showing clinical signs consistent with TeHVs infection should be isolated from clinically healthy individuals and tested for the presence of TeHVs (PCR, virus isolation) and for the exposure to the virus by ELISA or SNT. All clinical animals should also be treated with antiviral drugs and supportive therapy as appropriate. Serological tests needs to be repeated after 8 (ELISA) or 10-12 weeks (SNT) for all the tortoises showing clinical signs that tested negative at the first sampling. Clinically healthy exposed animals should be closely monitored and tested for viral exposure similarly to what described above.

Serologically and/or PCR positive animals that have recovered from the disease and clinically healthy animals that have seroconverted (and/or have tested positive by PCR) following viral exposure should be considered carrier for life. Currently, no evidence exists that supports vertical transmission of the virus.

Preventive measures include:

1) All the tortoises entering into established collections should be tested for the presence and exposure to the virus ideally both by molecular and serological tests. Viral isolation is also recommended. Animals should be tested during the quarantine period, which ideally should last no less than 6 months. Molecular diagnostic tests and viral isolation are relevant in the acute stage of the disease and during viral recrudescence when the virus is actively replicating. Serological tests are relevant especially after the acute stage of the disease,

TESTUDINID HERPESVIRUSES

during the latency of the virus, when seroconversion has occurred and no active viral replication can be detected. Testing an animal during the acute stage of the disease with serological tests only is likely to provide a false negative result, since seroconversion has not occurred yet. However, this is still recommended since some diseased animals might not be showing a primary infection, but differently a recrudescence of the infection and so they might have already seroconverted. Additionally, for naïve negative animals this would be considered the “pre-immune” serum. Clinically healthy infected animals that have survived the acute stage of the disease are likely to shed the virus only sporadically and this can determine a high rate of false negative animals if tested solely by PCR and/or virus isolation at that time. It is recommended to repeat two serological tests no less than 8 or 10-12 weeks apart when running the ELISA test or the SNT, respectively. Occasional “non reactors” do exist.

- 2) It is very important to avoid mixing of different tortoise species due to evidence of different species-specific resistances/sensitivity to TeHVs. Mixing of different species might be fatal for the less resistant species.
- 3) It is important to avoid overcrowding, since close contact between tortoises is considered to enhance the transmission of TeHVs.
- 4) Contaminated soil might contain viable virus for 2-4 up to 9-24 weeks according to the season of the year. Exposure to full sunlight of the contaminated soil might reduce the viability of the virus present.
- 5) Disposable gloves and shoe cover should be changed when moving from pen to pen and when touching different tortoises. An appropriate hands disinfectant is also recommended.
- 6) A veterinary health check should be performed ideally every 6 months. For tortoises from temperate climates a health check performed before and after brumation is critical.

Suggested disinfectant for housing facilities: Any proven virucidal compound may be used to disinfect contaminated instrument and/or pen furniture

Notification: TeHVs associated diseases are not reportable at the moment.

Measures required under the Animal Disease Surveillance Plan: N/A

Measures required for introducing animals to an infected animal(s): If a seronegative animal is introduced to a seropositive animal it should be expected that the seronegative animal will seroconvert eventually.

Conditions for restoring disease-free status after an outbreak: N/A

Experts who may be consulted:

Francesco C Origg, DVM, PhD, DACVM, DACVP
 Centre for Fish and Wildlife Health (FIWI)
 Vetsuisse Faculty, University of Bern
 Länggassstrasse 122, Bern-CH
 + 41 31 631 2443
 Fax. + 41 31 631 2635
 Francesco.origg@vetsuisse.unibe.ch

References:

1. Bicknese E.J., A.L. Childress, and J.F. Wellehan. 2010. A novel herpesvirus of the proposed genus Chelonivirus from an asymptomatic bowsprit tortoise (*Chersina angulata*). *J. Zoo Wildl. Med.* 41: 353–358.
2. Jacobson, E.R., K.H. Berry, J.F.X. Wellehan, F.C. Origg, A.L. Childress, J. Braun, M. Schrenzel, J. Yee, and B. Rideout. 2012. Serologic and molecular evidence for testudinid herpesvirus 2 infection in wild Agassiz’s desert tortoises, *Gopherus agassizii*. *J. Wildl. Dis.* 48: 747–757.
3. Marschang, R.E., M. Gravendyck, and E.F. Kaleta. 1997. Herpesviruses in tortoises: investigations into virus isolation and the treatment of viral stomatitis in *Testudo hermanni* and *T. graeca*. *Zentralbl.*

TESTUDINID HERPESVIRUSES

- Veterinarmed. B, 44:385–394.
4. Marschang, R.E., and R.M. Schneider. 2007. Antibodies to viruses in wild-caught spur-thighed tortoises (*Testudo graeca*) in Turkey. *Vet. Rec.* 161: 102–103.
 5. Marschang, R.E., J.W. Frost, M. Gravendyck, and E.F. Kaleta. 2001. Comparison of 16 chelonid herpesviruses by virus neutralization tests and restriction endonuclease digestion of viral DNA. *J. Vet. Med. B, Infect. Dis. Vet. Pub. Health*: 48: 393–399.
 6. Marschang, R.E., C.B. Gleiser, T. Papp, A.J. Pfitzner, R. Böhm, and B.N. Roth. 2006. Comparison of 11 herpesvirus isolates from tortoises using partial sequences from three conserved genes. *Vet. Microbiol.* 117: 258–266.
 7. Origgi, F.C. 2006. Tortoise Herpesvirus. *In: Mader, D.R. (ed): Reptile Medicine and Surgery.* WB Saunders Co., St. Louis, MO. Pp. 814–821.
 8. Origgi, F.C. 2012. Testudinid herpesviruses: a review. *J. Herpetologic Med. Surg.* 22: 42-54.
 9. Origgi, F.C., P.A. Klein, K. Mathes, S. Blahak, R.E. Marschang, S.J. Tucker, and E.R. Jacobson. 2001. Enzyme-linked immunosorbent assay for detecting herpesvirus exposure in Mediterranean tortoises (spur-thighed tortoise [*Testudo graeca*] and Hermann's tortoise [*Testudo hermanni*]). *J. Clin. Microbiol.* 39: 3156–3163.
 10. Origgi, F.C., C.H. Romero, D.C. Bloom, P.A. Klein, J.M. Gaskin, S.J. Tucker, and E.R. Jacobson. 2004. Experimental transmission of a herpesvirus in Greek tortoises (*Testudo graeca*). *Vet. Pathol.* 41: 50–61.
 11. VanDevanter, D.R., P. Warrener, L. Bennett, E.R. Schultz, S. Coulter, R.L. Garber, and T.M. Rose. 1996. Detection and analysis of diverse herpesviral species by consensus primer PCR. *J. Clin. Microbiol.* 34: 1666–1671.