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Short communication

Long-term monitoring of four Scutavirus testudinidalpha3-infected Mediterranean tortoises (Testudo spp.) from a 2013 outbreak: a case study

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Abstract

*Scutavirus testudinidalpha*3 (formerly known as Testudinid *alphaherpesvirus* 3) is a virus causing severe and ultimately fatal diseases in Mediterranean tortoises. The virus can alternate between a lytic phase, involving viral replication, and a latent stage. It is considered a threat for all the Testudinidae, including endangered species. Long-term outcomes of surviving animals post-primary infection remain unstudied. The aim of this work was to conduct an 8-year long-term monitoring study of a group of *Scutavirus testudinidalpha3*-infected Mediterranean tortoises, including two *Testudo hermanni hermanni* and two *T. graeca ibera*, that survived a high mortality-associated outbreak in 2013. Serological (ELISA) and molecular test (PCR from oral swab) were employed for the long-term monitoring of the infection on the four *Testudo* spp. *Scutavirus testudinidalpha3* DNA and anti-*Scutavirus testudinalpha3* antibodies were detected in three animals after 7- and 8-years post-outbreak, respectively. The fourth individual tested negative both by PCR and serology after an initial transient positivity recorded during the outbreak. Despite the limited sample size, the combination of clinical diagnosis and laboratory results assessed during investigation underscore the complexity and unpredictability of *Scutavirus testudinidalpha3* infection and its long-term outcomes. These findings should be considered in the management and exchange of tortoises, especially those from different origins.

Keywords

Scutavirus testudinidalpha3, Testudinid alphaherpesvirus 3, Testudo hermanni, Testudo graeca, long-term monitoring

Introduction

Scutavirus testudinidalpha3 (formerly known as *Testudinid alphaherpesvirus 3*, Family Orthoherpesviridae, subfamily Alphaherpesvirinae, Genus *Scutavirus*, based on the International Committee on Taxonomy of Viruses, ICTV, 2023) is a virus that induces severe and often fatal diseases among a broad spectrum of tortoises, predominantly in Mediterranean species (*Testudo graeca*, *T. hermanni*, *T. marginata*) and *T. horsfieldii*. It also affects other species like *Centrochelys sulcata*, *Astrochelys radiata, Stigmochelys pardalis*, and *Chelonoidis carbonarius* (Origgi, 2012; Okoh et al., 2021). There is a distinct degree of susceptibility among species, leading to variability in disease severity and mortality rates. Notably, lethality is disproportionately higher in *T. hermanni* (75% and more) compared to *T. graeca* (Origgi et al., 2001; Origgi, 2012; Marenzoni et al., 2018; Leineweber et al., 2021).

T. graeca, commonly known as the Greek tortoise, is native to North Africa, Southern Europe, and parts of the Middle East. *T. graeca ibera*, also known as the Iberian tortoise, is a subspecies of *T. graeca* and is found in southeastern regions of Europe. Both these subspecies are classified as vulnerable on the Red List of the International Union for Conservation of Nature (IUCN), primarily due to habitat loss and illegal collection. *T. hermanni,* or Hermann's tortoise, is distributed in the northern Mediterranean area and has two subspecies: *T. hermanni hermanni*, found in the western Mediterranean from Catalonia to Italy, and the eastern subspecies, *T. hermanni boettgeri*, inhabiting the Balkans and Greece. *T. hermanni* is considered near-threatened according to the IUCN Red List, while the Italian Red List Reports

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T. h. hermanni as endangered (Rondinini et al. 2022). Human activities, including habitat loss and illegal collection of wild individuals, heavily contribute to the decline of this species. Accordingly, it is protected by national and international laws, with efforts to monitor populations and manage smuggled or confiscated individuals supported by the Italian Ministry of the Environment (MATTM, 2019). *T. marginata*, the Marginated tortoise, native to Greece and naturalized on Sardinia Island, is considered a stable population according to the IUCN Red List (MATTM, 2019).

The clinical disease typically evolves from initial infection to acute clinical signs such as respiratory distress, lethargy, and loss of appetite. These clinical signs can progress to death in the most susceptible species. In many cases, the infection progresses to a chronic state alternating with a dormant, clinically silent stage (latency), allowing clinically healthy or subclinically infected hosts to potentially disseminate the virus within a collection of animals (Origgi, 2012). Consequently, the virus poses a significant threat to tortoise populations, particularly those listed as endangered, in which it causes mortality (Marenzoni et al 2018; Marschang et al 2009; Origgi et al., 2001, 2004; Origgi, 2012). The trade and exchange of individuals among breeders without appropriate testing is an unfortunate practice, which presents a serious risk for viral transmission and should be strongly discouraged (Marenzoni et al., 2018; Leineweber et al., 2021; Rush et al., 2021).

Despite numerous documented *Scutavirus testudinidalpha3* outbreaks, there is virtually no knowledge concerning the long-term outcome of these outbreaks in tortoise collections, including survival rate, the extent of viral shedding, and clinical disease re-occurrence. Particularly, there is scant evidence concerning infection dynamics in highly susceptible *T. hermanni* individuals who have survived previous outbreaks. This information is relevant to understanding and foresee the conditions of the reactivation and spread of the *Scutavirus testudinidalpha3* infection over time and space.

To address this knowledge gap, a long-term monitoring study was carried out on a small cohort of *Scutavirus testudinidalpha3*-infected Mediterranean tortoises (two *T. hermanni hermanni* and two *T. graeca ibera*) that had survived an outbreak 8 years prior (Marenzoni et al., 2018). The study's objective was to determine the long-term survival rates, patterns of viral shedding, and the potential for clinical disease re-occurrence in these survivors. Insights into the persistence and reactivation of the virus, as well as its transmission dynamics, are crucial for developing effective management and conservation strategies for these endangered tortoise species.

Materials and methods

Background of the 2013 Scutavirus testudinidalpha3 outbreak

During the 2013 outbreak of *Scutavirus testudinidalpha3*, the facility had 314 chelonians, including 252 *T. hermanni hermanni*, 5 *T. hermanni boettgeri*, 7 *T. graeca ibera*, 4 *Testudo horsfieldii*, 3 *T. marginata*, 41 turtles (16 *Trachemys* spp., 14 *Emys orbicularys*, 5 *Graptemys* spp., 4 *Pseudemys* spp., 2 *Cuora amboinensis*) and 3 *Terrapene carolina mayor*. The facility experienced the loss of 15 *T. hermanni,* with a morbidity of 5.5% (15/270), a mortality 5.5% (15/270), and a case-fatality of the 75% (15/20). At the end of the observation period (December 2014), only four exposed and infected tortoises survived: two *T. hermanni hermanni* (case 1 and case 3) and two *T. graeca ibera* (case 2 and case 4). These four individuals remained free of clinical signs throughout the outbreak (Marenzoni et al., 2018).

During the outbreak, ill, suspected infected, exposed, and suspected contaminated individuals were evaluated, where possible, using ELISA tests to search for antibodies and PCR methods on oral swabs from live animals and tissues from deceased animals, specifically to detect *Scutavirus testudinidalpha3* (Marenzoni et al., 2018). A total of 22 subjects were analysed multiple times over the course of the outbreak. Following the outbreak, these surviving animals were kept in isolation, separated from the other tortoises. Throughout 2014, the same individuals underwent repeated checks to assess their *Scutavirus testudinidalpha3* status and the occurrence of detectable viral shedding (Marenzoni et al., 2018).

In October 2015, all animals in the collection, including the four that were in isolation, were moved to another location. March 2016 was the first post-hibernation period after their transfer to the new housing, where they had been housed until the end of the study.

Current population and housing conditions during the study

At the time of the study, after the 2013 outbreak and during the monitoring, the breeding facility contained approximately 450 chelonians. The population included 278 *T. hermanni hermanni*, 20 *T. hermanni boetgerii,* 66 *T. graeca ibera*, 9 *T. horsfieldii*, 42 *T. marginata*, and 43 turtles from various species including *Cuora* spp., *Emys orbicularis*, *Graptemys* spp., *Kinosternon* spp., *Mauremys* spp., *Pseudemys* spp., *Terrapene* spp., and *Trachemys*

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spp. All animals were housed outdoors and divided into small groups based on species and age. The adult tortoises were placed in nearly 20 separate fenced enclosures.

Sampling

Owner was asked to fill in consent forms permitting the use of data collected during diagnostic evaluations. No ethical permission was required for the sampling because all procedures presented in this study were performed solely for diagnostic purposes in infected and suspected infected animals.

Oral swabs (OS) and blood samples were collected from the tortoises at various time points throughout the study. For logistical reasons, it was not always possible to perform annual sampling, and in some cases of the samplings, some subjects were not compliant. In 2014, the first year post-outbreak, samples were taken in the spring (April), summer (June), autumn (October), and winter (November). In 2015, sampling occurred during the pre-hibernation period in October, immediately after the animals were translocated. In 2016, samples were collected during the post-hibernation period, following the translocation of the animals. In 2020, sampling was again performed during the post-hibernation period. In 2021, sampling was carried out during the post-hibernation period, specifically on March 31, and repeated on April 5.

Serological tests were performed during the post-hibernation of 2013, 2019, and 2021.

OS were collected by rotating sterile polyester swabs, dipped in 500 µL of phosphate-buffered saline (PBS, pH 7.2), against the mucosa of the mouth. All swabs were stored at 4 °C in PBS until analysis, which was performed within 24–48 h.

Blood samples were collected from the cervical dorsal sinus using sterile, plain Vacutainer tubes (Becton Dickinson, Milan, Italy). Sera were obtained by centrifugation at 6,000x*g* for 1 min.

PCR

Two hundred µL of OS transport buffer (PBS) were used for DNA extraction, applying a commercial kit (DNeasy Tissue kit, Qiagen, Italy). The concentration and purity of the extracted DNA were quantified using a microvolume spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Italy). For the PCR analysis, 10 µL of DNA extract solution was tested. This included a nested, consensus, pan-herpesvirus PCR targeting the partial sequence of the DNA polymerase gene (Vandevanter et al., 1996) to cover the entire range of possible herpesviruses existing in *Testudo*; additionally, a semi-nested *Scutavirus testudinidalpha3* (genotypic)-specific PCR (Origgi et al., 2004; Marenzoni et al., 2018) was conducted to detect *Scutavirus testudinidalpha3* specifically. After the initial screening using the consensus pan-herpesvirus PCR protocol to identify any herpesvirus in the tortoises, it was discontinued in favor of the semi-nested protocol *Scutavirus testudinidalpha3* (genotypic)-specific PCR, which is six times more sensitive and therefore preferred for subsequent diagnostic purposes.

Serological assay

An ELISA test, validated for the detection of anti-*Scutavirus testudinalpha3* antibodies in *T. hermanni* and *T. graeca*, was carried out according to standard procedures (Origgi et al., 2001; Origgi et al., 2004; Marenzoni et al., 2018), using plasma obtained from the animals.

Results

In addition to the long-term monitoring of the four tortoises post-2013 outbreak, findings from the 2013*Scutavirus testudinidalpha3* outbreak provide a critical context for the current study. The outbreak was documented and analyzed in detail in a previous publication (Marenzoni et al., 2018), revealing key insights into viral dynamics and transmission patterns. The observation period of the outbreak, as described by Marenzoni et al. (2018), extends until December 2014. During this outbreak, which began in March 2013, the facility lost 15 *T. hermanni*. Thirteen of these tortoises (9 T*. hermanni hermanni* and 4 *T. hermanni boettgeri*) died within the first two months of the outbreak, while two more T *hermanni hermanni* died during the post-hibernation period in March 2014, the year after. Additionally, a *T. marginata*, likely the carrier of the infection in the facility, died in August 2014. In this case, necropsy did not reveal any lesions, PCR from tissues was negative for *Scutavirus testudinidalpha3*, so its death was not considered directly caused by the infection. At the end of the outbreak observation period (December 2014), only four exposed and infected tortoises survived: two *T. hermanni hermanni* (case 1 and case 3) and two *T. graeca ibera* (case 2 and case 4).

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ID animal	Species	Sex	Age (years)	PCR results	PCR results 2014				PCR results 2015	PCR results 2016	PCR results 2019	PCR results 2020	PCR results 2021	PCR results 2021
					Apr	June	Oct	Nov	October	March		March	(31 March)*	(5 April)*
case 1	*T. hermanni hermanni*	female	>30	+	+	-	-	-	-	-	np	-	-	-
case 2	*T. graeca ibera*	female	>30	+	np	+	-	np	-	-	np	-	+	-
case 3	*T. hermanni hermanni*	female	18	+	np	-	-	-	-	-	np	+	-	-
case 4	*T. graeca ibera*	male	24	+	np	+	+	np	-	-	np	+	-	-

np: not performed

*note that the sampling was performed after five days.

Table I. Scutavirus testudinidalpha3 PCR monitoring of the four Scutavirus testudinidalpha 3-infected Mediterranean tortoises from 2013 to 2021. The results refer to the specific semi-nested PCR from oral swabs of the animals.

ID animal	Species	ELISA results 2013 outbreak	ELISA results 2019	ELISA results 2021
case 1	*T. hermanni hermanni*	+	-	-
case 2	*T. graeca ibera*	np	+	+
case 3	*T. hermanni hermanni*	np	+	+
case 4	*T. graeca ibera*	+	+	np

Table II. Scutavirus testudinidalpha3 serological monitoring of the four Scutavirus testudinidalpha3-infected Mediterranean tortoises from 2013 to 2021. np: not performed.

For the present study, OS and blood samples were collected from the survived tortoises at various time points to comprehensively understand the outcome of the *Scutavirus testudinidalpha3* infection in the surviving *Testudo* species, specifically to monitor any ongoing viral shedding and assess the long-term health and infection status of these individuals. The four tortoises remained alive (100% survival rate) showed no obvious clinical signs throughout the entire monitored period, including the post-hibernation seasons. The results of the molecular (from OS) and serological (from plasma) tests for detecting the presence of *Scutavirus testudinalpha3* DNA and exposure to the virus (ELISA) between 2014 and 2021 are summarized in Tables I and II.

Samples positive to PCR revealed a weak amplification in the first step of the protocol (Origgi et al., 2004), but demonstrated strong positivity following the semi-nested protocol (Marenzoni et al., 2018). However, these samples were negative using the pan-herpesvirus PCR, which was initially used as a screening test to identify any testudinid herpesviruses.

There is evidence of viral shedding in at least three out of the four subjects. A fourth subject, which did not display any clinical signs but was PCR- and ELISA-positive during the outbreak, consistently tested negative in subsequent years.

Discussion

Herpesviral infections persist for life. A positive PCR test indicates the presence of viral genomic material in the host, while an initial positive serological test suggests antigenic exposure. One of the tortoises (case 1) in this study showed a transient molecular and serological positivity during the outbreak, followed by consistently negative test results later. It remains uncertain whether this occurrence resulted from an abortive infection or from exposure that did not lead to a sustained viral replication. Studies in *T. graeca* have demonstrated that even after exposure to a significant viral load (1.5×10^5 $TCID_{50}$), seroconversion may not necessarily occur, and the viral colonization within the individual might be extremely limited (Origgi et al., 2004). Furthermore, in the absence of a full necropsy, which is not feasible in live animals as in the current study, it is not possible to determine whether a negative result reflects a complete absence of the virus or simply a lack of shedding, especially when relying solely on swab sampling, as in the current scenario. The absence of antibody detection after an initial positive test might be attributed to the known fluctuations in antibody

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titers among infected individuals. In this case, these fluctuations might not have been boosted by subsequent viral reactivations.

Viral shedding was detected in the other three individuals 7 and 8 years after the initial outbreak.

The detection of viral DNA using a PCR protocol that is six times more sensitive (Marenzoni et al., 2018) compared to the widely employed consensus PCR protocol for Herpesviridae (vanDevanter et al., 1996) is not surprising, given that consensus PCRs are often characterized by low sensitivity. However, a relatively low viral load in the sample cannot be ruled out. Furthermore, in 2021, one tortoise (case 2) tested negative only 5 days after a previous sampling, which yielded a positive result. This is consistent with sporadic viral shedding, a phenomenon that can occur in infected but clinically healthy individuals. This closely aligns with observations in other species, including humans and horses during herpesvirus infections, such as the human herpes simplex virus or equine herpesvirus 1, which can be transmitted even in asymptomatic cases for as little as 1 day (Wald et al., 1995; Pusterla et al., 2024). Consequently, conducting multiple samplings, in close temporal proximity, in animals infected with *Scutavirus testudinalpha3* is essential for future studies to better understand infection dynamics and identify infected individuals without clinical signs.

Viral shedding appeared to be strongly influenced by seasonality, particularly post-hibernation, aligning with previous observations (Origgi et al., 2015; Marenzoni et al., 2018; Leineweber et al., 2021). Additional factors, other than seasonality, are likely to play a relevant role in the viral shedding of *Scutavirus testudinalpha3*; among these factors, stress—potentially linked to events such as translocation, changes in environmental conditions, social dynamics within the group, or reproductive cycles—could be significant. These stressors can affect the immune system of the tortoises, potentially increasing their susceptibility to viral shedding (Zapata et al., 1992; French et al., 2008; Zimmermann et al., 2010; Fazio et al., 2014). Nevertheless, no virus was detectable shortly after the tortoises were relocated in the current study, suggesting that translocation was not a significant stressor, as observed in previous studies (Anderson et al., 2015). This implies that other, less understood, factors might also influence viral shedding dynamics.

The other issue to consider in assessing the translocation as a possible viral reactivator might be the need to perform repeated and closely timed sampling, as previously mentioned. The apparent random pattern of viral shedding during a phase of active viral replication resulted in the present study, could potentially account for the failure to detect the virus when systematic, short-term sampling was not in place.

The persistency of the virus in the infected individuals, associated with a complete unpredictability of viral shedding, even during the most likely periods to occur (such as post-hibernation), over several years, and in the absence of visible lesions, poses a significant threat to the management of both pet and free-ranging populations. The persistence status of viral shedder observed in three out of four tortoises suggests a high prevalence of shedders among herpesvirus infected tortoises, despite the limitations of observing such a small cohort. To mitigate this threat, it is crucial to implement stringent biosecurity measures, including routine health monitoring and testing of both symptomatic and asymptomatic individuals. Isolating infected tortoises, especially during periods of high viral shedding like post-hibernation, can help prevent the spread of the virus. Additionally, educating pet owners and wildlife managers about the importance of quarantine procedures for new or returning animals can reduce the risk of introducing or reintroducing the virus into populations (Marenzoni et al., 2018; MATTM, 2019).

Awareness among owners and field biologists is necessary to limit the diffusion of this virus within naïve individuals and populations, especially endangered species, like *T. hermanni,* which notoriously is highly sensitive to the infection (Marenzoni et al., 2018). The risks for *T. hermanni* include severe morbidity and high mortality rates due to the virus, which could lead to significant population declines, especially dangerous in a natural population. Screening tests are necessary and should be performed whenever animals are expected to be relocated or introduced into new collections, associated with the quarantine of new arrivals, which, however, may not be sufficient (Marenzoni et al., 2018).

Post-hibernation testing appears to be ideal timing for detecting the largest number of infected individuals due to reduced immune defenses following the hibernation period (Muñoz et al., 2000; Zimmermann et al., 2010; Marenzoni et al., 2018), which may result in increased viral shedding and higher detectability. Employing repeated sampling with complementary tests (ELISA and PCR) to identify infected individuals remains the most effective and recommended preventive strategy to mitigate the viral spread.

This data underscores the critical need for regular monitoring and early detection to prevent outbreaks. Future studies should focus on clarifying the specific dynamics of the infection, including the mechanisms behind seasonal variations in immune response and viral shedding. Ongoing research into viral behaviour and the development of tools to contrast the virus (potential vaccines or antiviral treatments) could also play a key role in managing this persistent threat, especially in captivity. Additionally, while proper management practices, including the isolation of infected individuals and the quarantine of new arrivals, are essential for controlling the spread of the virus in captive settings,

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similar strategies and further research are equally important for protecting wild animal populations and preserving their health in natural environments.

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Author contributions

Maria Luisa Marenzoni: conceptualization, methodology, investigation, formal analysis, writing original draft, writing review and editing, supervision. Lorenzo Santoni: conceptualization, methodology, writing original draft, writing review. Elisa Rossi: formal analysis, writing review and editing. Francesco Carlo Origgi: conceptualization, methodology, investigation, formal analysis, writing original draft, writing review and editing, supervision.

Competing interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Anderson, L. E., Cree, A., Towns, D. R., & Nelson, N. J. (2015). Moving house: long-term dynamics of corticosterone secretion are unaltered in translocated populations of a rare reptile (the tuatara, Sphenodon punctatus). Conservation physiology, 3(1), cov014.

Fazio, E., Medica, P., Bruschetta, G., & Ferlazzo, A. (2014). Do handling and transport stress influence adrenocortical response in the tortoises (Testudo hermanni)?. International Scholarly Research Notices, 2014(1), 798273.

French, S. S., Fokidis, H. B., & Moore, M. C. (2008). Variation in stress and innate immunity in the tree lizard (Urosaurus ornatus) across an urban–rural gradient. Journal of Comparative Physiology B, 178, 997-1005.

ICTV (International Committee on Taxonomy of Viruses). (n.d.). Scutavirus testudinidalpha3. Retrieved from https://ictv.global/taxonomy/taxondetails?taxnode_id=202206398&taxon_name=Scutavirus%20testudinidalpha3

Leineweber, C., Müller, E., & Marschang, R. E. (2021). Herpesviruses in captive chelonians in Europe between 2016 and 2020. Frontiers in Veterinary Science, 8, 733299. https://doi.org/10.3389/fvets.2021.733299

MATTM, Ministero dell'ambiente e della tutela del territorio e del mare (2019). Linee guida per il ricollocamento in natura di individui del genere Testudo spp. confiscati. Available at: https://www.minambiente.it/sites/default/files/archivio/allegati/cites/linee_guida_testudo_aggiornate_2019.pdf, accessed 27 July 2024.

Marenzoni, M. L., Santoni, L., Felici, A., et al. (2018). Clinical, virological and epidemiological characterization of an outbreak of Testudinid Herpesvirus 3 in a chelonian captive breeding facility: Lessons learned and first evidence of TeHV-3 vertical transmission. PLoS One, 13(5), e0197169. https://doi.org/10.1371/journal.pone.0197169

Marschang, R. E., Papp, T., Ferretti, L., et al. (2009). Detection and partial characterization of herpesviruses from Egyptian tortoises (Testudo kleinmanni) imported into Italy from Libya. Journal of Zoo and Wildlife Medicine, 40, 211-213.

Muñoz, F. J., Galván, A., Lerma, M., & De la Fuente, M. (2000). Seasonal changes in peripheral blood leukocyte functions of the turtle Mauremys caspica and their relationship with corticosterone, 17-β-estradiol and testosterone serum levels. Veterinary Immunology and Immunopathology, 77(1-2), 27-42.

Okoh, G. R., Horwood, P. F., Whitmore, D., et al. (2021). Herpesviruses in reptiles. Frontiers in Veterinary Science, 8, 642894. https://doi.org/10.3389/fvets.2021.642894

Origgi, F. C. (2015). A genomic approach to unravel host-pathogen interaction in chelonians: The example of Testudinid Herpesvirus 3. PLoS One, 10(8), e0134897. https://doi.org/10.1371/journal.pone.0134897

Origgi, F. C., Klein, P. A., Mathes, K., et al. (2001). Enzyme-linked immunosorbent assay for detecting herpesvirus exposure in Mediterranean tortoises (spur-thighed tortoise [Testudo graeca] and Hermann's tortoise [Testudo hermanni]). Journal of Clinical Microbiology, 39(9), 3156-3163. https://doi.org/10.1128/JCM.39.9.3156-3163.2001

Origgi, F. C., Romero, C. H., Bloom, D. C., et al. (2004). Experimental transmission of a herpesvirus in Greek tortoises (Testudo graeca). Veterinary Pathology, 41(1), 50-61. https://doi.org/10.1354/vp.41-1-50

Pusterla, N., Lawton, K., & Barnum, S. (2024). Investigation of the Use of Environmental Samples for the Detection of EHV-1 in the Stalls of Subclinical Shedders. Viruses, 16(7), 1070. https://doi.org/10.3390/v16071070

Rondinini, C., Battistoni, A., Teofili, C. (2022) Lista Rossa IUCN dei vertebrati italiani 2022. Comitato Italiano IUCN e Ministero dell'Ambiente e della Sicurezza Energetica, Roma

Rush, E. R., Dale, E., & Aguirre, A. A. (2021). Illegal Wildlife Trade and Emerging Infectious Diseases: Pervasive Impacts to Species, Ecosystems and Human Health. Animals (Basel), 11, 1821.

VanDevanter, D. R., Warrener, P., Bennett, L., et al. (1996). Detection and analysis of diverse herpesviral species by consensus primer PCR. Journal of Clinical Microbiology, 34(7), 1666-1671. https://doi.org/10.1128/JCM.34.7.1666-1671.1996

Wald, A., Zeh, J., Selke, S., et al. (1995). Virologic characteristics of subclinical and symptomatic genital herpes infections. New England Journal of Medicine, 333(12), 770-775. https://doi.org/10.1056/NEJM199509213331205

Zapata, A. G., Varas, A., & Torroba, M. (1992). Seasonal variations in the immune system of lower vertebrates. Immunology today, 13(4), 142-147.

Zimmerman, L. M., Vogel, L. A., & Bowden, R. M. (2010). Understanding the vertebrate immune system: insights from the reptilian perspective. Journal of Experimental Biology, 213(5), 661-671.