Received: 26 August 2021 | Revised: 27 October 2021 | Accepted: 7 November 2021

DOI: 10.1111/tbed.14391

Transboundary and Emerging Diseases

WILEY

ORIGINAL ARTICLE

Health assessments uncover novel viral sequences in five species of Galapagos tortoises

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Funding information
Saint Louis Zoo Institute for Conservation Medicine; Saint Louis WildCare Institute Center for Chelonian Conservation; American Association of Zoo Veterinarians Wildlife Animal Health Fund:, Grant/Award Number: WAHF #37; Houston Zoo; Galapagos Conservation Trust; Charles Darwin Foundation; Ecoventura; Miss Linda Esler

Abstract

Emerging infectious diseases (EIDs) have been reported as causes of morbidity and mortality in free-living animal populations, including turtles and tortoises, and they have even resulted in species extinctions, with human activities contributing to the spread of many of these diseases. In the Galapagos, giant tortoises are endangered due to habitat change, invasive species, and other human impacts; however, the impact of EIDs on Galapagos tortoise conservation remains understudied. To fill this gap, we conducted health assessments of five tortoise species from the islands of Santa Cruz, Isabela and Española. We performed health evaluations of 454 animals and PCR testing for pathogens known to be relevant in other tortoise species. We identified two novel sequences of adenoviruses and four of herpesviruses. Based on alignments of the DNA polymerase gene and maximum likelihood phylogenetic analyses, we found both novel adenoviruses to be most closely related to red footed tortoise adenovirus 2, by nucleotide sequence and red footed tortoise adenovirus 1, based on amino acid sequence. Three of the herpesvirus sequences translated into the same deduced amino acid sequence; therefore, they may be considered the same viral species, closely related to terrapene herpesvirus 2. The fourth herpesvirus sequence was highly divergent from any sequence previously detected and is related to an eagle owl herpesvirus based on nucleotide sequence and to loggerhead oro-cutaneous herpesvirus based on amino acids. These novel viruses may be pathogenic for giant tortoises under specific conditions (e.g., stress). Continued screening is crucial to determine if these viruses play a role in tortoise fitness, morbidity and survival. This information allows us to provide recommendations to the Galapagos National Park Directorate and other institutions to improve the management of these unique species both in Galapagos and globally, and for tortoise reintroduction plans throughout the archipelago.

KEYWORDS
adenovirus, *Chelonoidis* spp, conservation medicine, herpesvirus, wildlife surveillance

1 | INTRODUCTION

Turtles and tortoises compose a taxonomic group that includes more than 300 species that live on land, in freshwater systems, and in oceans; however, this large group of vertebrates is among the most threatened according to the IUCN Red List criteria (Rhodin et al., 2018; Stanford et al., 2020). The combined effects of habitat loss and degradation, consumption and use of the animals and their eggs, invasive species and climate change are bringing many of the world's tortoises and turtles to the brink of extinction, and Galapagos tortoises are no exception. Two Galapagos tortoise species became extinct in the last two centuries due to human predation, and all of the twelve extant species are considered threatened with extinction (IUCN, 2020). New challenges for Galapagos tortoise conservation include the dispersion of antimicrobial resistance genes into the environment driven by human activities (Nieto-Claudin et al., 2021a) and the impacts of plastics and pesticides, which are largely under-recognised based on our initial studies. Emerging infectious diseases (EIDs) also have been reported as causes of morbidity and mortality of free-living animal populations including tortoises; however, disease and pathogens are not usually listed as potential or current threats to reptile populations, as they remain poorly understood (Daszak et al., 2000; Gibbons & Steffes, 2013). Recent studies highlight the importance of expanding EID studies in turtles and tortoises to prevent mortality events of already compromised and declining populations of high conservation concern (Doszpoly et al., 2013; Kane et al., 2017; Kolesnik et al., 2017). In the Galapagos Archipelago, giant tortoises play key roles for maintaining healthy ecosystems (e.g., seed dispersal, habitat modifications) while also significantly contributing to the local economy based on eco-tourism activities, as these giants are considered one of the main attractions for visitors and researchers (Benitez-Capistros et al., 2019; Blake et al., 2012). Since the late 1970s, the reintroduction of Galapagos tortoises in captive-breeding and restoration programs have resulted in hundreds of tortoises moved across the archipelago (Gibbs et al., 2014), with the potential to disseminate and/or introduce pathogens to naïve populations, as has occurred in other animal species and countries with rabies and bovine brucellosis (Fèvre et al., 2006; Massei et al., 2010). Despite their importance, infectious agents that might be present in free-living giant tortoises and their potential impacts on tortoise and reptile conservation remain understudied.

Research on reptile virology and microbiology has undergone rapid expansion over the past few decades. Interest in reptile-specific pathogens has focused on both their importance to reptile medicine and conservation, as well as on increasing knowledge of microbe systematics and evolution (Agius et al., 2019). Several infectious diseases continue to be prevalent in captive and free-living chelonians, including the well-documented mycoplasmosis and herpesvirus-related diseases, whereas other pathogens are currently emerging in tortoises (i.e., adenovirus, ranavirus) (Gibbons & Steffes, 2013; Marschang, 2011). Concurrent infections with these pathogens have been described and may lead to more severe systemic disease and mortality in turtles experiencing these co-infections (Kolesnik et al., 2017; Sim et al., 2016).

Herpesvirus (HV) infections have been reported in many different species of tortoises (Testudinidae), including captive and free-living populations (Kane et al., 2017; Origgi, 2012; Sim et al., 2015; Yonkers et al., 2015). Clinical signs commonly associated with HV infections range from subclinical to severe, including rhinitis, conjunctivitis, stomatitis and glossitis, which frequently develop into a diphtheroid-necrotising process with diphtheroid membranes covering parts of the oral cavity and extending down into the trachea and oesophagus (Marschang, 2019). Oedema 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 (Marschang, 2019). Adenoviruses have only relatively recently been detected in several species of chelonians, showing a high mortality rate in some species such as Sulawesi tortoises (Rivera et al., 2009). Pathological findings in infected tortoises may include stomatitis, esophagitis, hepatic necrosis and lipidosis, myeloid necrosis in bone marrow, necrotising enterocolitis, pneumonia and encephalitis (Gibbons & Steffes, 2013; Marschang, 2011). Tortoise mycoplasmosis is one of the most extensively characterised infectious diseases of chelonians and has been associated with population declines in free-living tortoises (Jacobson et al., 2014). Clinical signs include lethargy, palpebral oedema, conjunctivitis and nasal and ocular discharges, causing significant upper respiratory tract disease in turtle and tortoises (Palmer et al., 2016). Ranaviruses have been increasingly shown to be important pathogens of ectothermic animals (Chinchar, 2002), regularly isolated from reptiles since the late 1990s, mostly in chelonian species. Ranavirus is associated with lethargy, anorexia, nasal and ocular discharge, conjunctivitis, severe subcutaneous cervical oedema, ulcerative stomatitis, 'red-neck disease' and death. Analysis suggests that reptilian ranavirus is originally transmitted from amphibians to reptiles; however, it has been demonstrated that it can also be transmitted between tortoise species (Marschang, 2011; Marschang et al., 2008).

For the current study, we designed a broad health assessment of giant tortoises across islands and species to test for four pathogens of conservation concern in tortoises; that is, herpesvirus, adenovirus, ranavirus and mycoplasmosis. Our goal was to describe the presence and prevalence of these potential pathogens within giant tortoise populations, their potential pathogenicity and if their presence might compromise tortoise conservation and well-being. This research advances knowledge of infectious agents in free-living Galapagos tortoises across species and islands.

2 | MATERIALS AND METHODS

2.1 | Study sites

We conducted the study on three islands of the Galapagos Archipelago, covering five different species of giant tortoises. Santa Cruz, located in the centre of the archipelago and inhabited by humans (S00.66551, W090.357241), contains two species of critically endangered giant tortoises, with the most predominant (*Chelonoidis porteri*) inhabiting

the central and southwestern area, and the other (*Chelonoidis donfaustoi*) restricted to the northeastern area of the island. The estimated population for *C. porteri* based on IUCN data from 2010 is 3400 individuals (Cayot et al., 2017), but no census has been conducted in the last decade. Through our work, we estimate a population that currently exceeds 6000 individuals (Blake, Cabrera, Nieto-Claudin, Deem unpublished data). The population of *C. donfaustoi* is estimated to be 550 individuals based on the latest census in 2018 (Tapia, 2018) and is believed to be one of the smallest population sizes for any Galapagos tortoise species.

Española Island is located in the south-east of the archipelago (S01.371006, W089.670863). This arid and small island is not inhabited by humans and contains one species of giant tortoise (*Chelonoidis hoodensis*) considered critically endangered by the IUCN (Cayot et al., 2017). After five decades of an intense captive-breeding program to restore its population, *C. hoodensis* is considered stable, and the last reproductive individuals maintained in captivity were released into the wild in June 2020 (Tapia, 2021a).

Isabela Island is the largest island, located on the eastern side of the archipelago.

Because of the isolating environment of each of its five volcanoes, a unique species of giant tortoise has evolved on each volcano. *Chelonoidis vandenburghi* is restricted to Alcedo Volcano and its slopes (S00.4409454°, W091.1068907°), and *Chelonoidis becki* is restricted to Wolf Volcano (S00.016080, W091.350429). A recent census conducted in Alcedo estimates its population to be 12,000–15,000 individuals (Tapia, 2021b), whereas the population of giant tortoises from Wolf is around 10,000–12,000 (Arteaga & Guayasamin, 2020). Alcedo and Wolf volcanoes have never been inhabited by humans, although an extensive goat eradication program to control for invasive species was carried out between 2004 and 2007 as part of the International Project Isabela (Lavoie et al., 2007). Since then, human activity on Alcedo and Wolf volcanoes has been restricted to scientific activities, with few scientists and rangers occasionally visiting every year.

2.2 | Sampling design and sample collection

Most samples were collected as part of a long-term health assessment within the Galapagos Tortoise Movement Ecology Programme (GTMEP) (Blake, Yackulic et al., 2015). From 2017–2020, we collected samples from 208 free-living western Santa Cruz tortoises, 55 from eastern Santa Cruz, 70 from Alcedo Volcano, and 45 from Española Island. In collaboration with Galapagos Conservancy and the Galapagos National Park Directorate, we also collected samples from 55 free-living Wolf tortoises. A total of five hybrids from *C. becki* and 16 tortoises from Española Island (*C. hoodensis*) maintained in captivity at the Fausto Llerena Breeding Center (Santa Cruz Island) were sampled and included in the present work. Samples collected from western Santa Cruz and Alcedo Volcano were also used in a broader study to assess antimicrobial resistance and reference intervals. For these two species, sample size was calculated based on the literature ($Z = 1.96$; $p = .05$) (Lwanga & Lemeshow, 1991). We also considered the feasibility of collecting the desired number of samples from a remote and isolated volcano such as Alcedo. Because of the logistical challenges of collection, for these tortoise species, sampling was opportunistic and took place in a single trip of 3–7 days, so all the samples were collected over a very short period of time and during the same season.

For each individual, we recorded morphometrics, weight and determined the sex in mature animals based on tail length and plastron concavity, as well as a body condition index (BCI) as previously described in Nieto-Claudin et al. (2021b) and Blake, Guézou et al. (2015). We identified tortoises by microchips previously placed by Galapagos National Park Service rangers. If no microchip was detected, we placed a new subcutaneous microchip (DATAMARS®) in the caudo-ventral area of the left hind leg. We collected conjunctival, oral and cloacal swabs using a sterile cotton swab (Puritan Sterile Products Company LLC, Maine, USA) and placed each in 2 ml sterile cryovials with no media. We kept all samples frozen at −30°C for up to 3 months until analyses.

We collected samples under the Galapagos National Park annual research permits PC-36-17, PC-35-18, PC-16-19, PC-28-20 and the International Animal Care and Use Committee from GREFA (Spain) with registration number 17/001. Samples from Fausto Llerena Breeding Centre were collected under the supervision of the Galapagos National Park Directorate. We transported frozen samples under exportation permits 153-2019-EXP-CM-FAU-DNB/MA, 192-2019-EXP-CM-FAU-DNB/MA and 031-2020-EXP-CM-FAU-DNB/MA.

2.3 | DNA extraction and molecular analysis

We added 100 μl of sterile PBS to each swab and allowed them to thaw for an hour at room temperature before carrying out the DNA extraction. We performed total DNA extraction directly from swab samples following the manufacturer's instructions (DNeasy Blood & Tissue Kit, Qiagen, Valencia, CA, USA). All DNA extraction occurred at the Charles Darwin Research Station (CDRS) in Galapagos, Ecuador.

For initial adenovirus screening, we used a nested PCR method with consensus primers targeting a 330 bp region of the DNA-dependent DNA polymerase gene of adenoviruses (Wellehan et al., 2004). We tested all extracted DNA from cloacal swabs at the CDRS. PCR products were resolved in 1.5% agarose gels. We used Sanger sequencing for all putative positive results using both primers. We then designed new degenerate primers (ADV_Chelonoidis-F: CTTCCAGGRCCTCCWCT; ADV_Chelonoidis-R: CGTCGACGGAGGTGATGA) from compatible bands obtained from the conventional PCR for use in a real-time PCR (qPCR) assay to test all samples. We used qPCR based on SYBR® Green dye, which amplifies a 209 bp fragment of the polymerase gene. A 20 μl reaction mixture consisted of 4 μl extracted DNA, 0.8 μl forward primer (20 μM), 0.4 μl reverse primer (20 μM) and 10 μl Kappa Master Mix (Sigma Aldrich, Spain). Reactions were amplified in a thermal cycler with an initial denaturation at 95°C for 3 min, followed by 40 cycles of amplification at 95°C for 15 s and 60°C for 30 s.

To test for herpesviruses in conjunctival and oral swabs from 454 tortoises, we used a consensus PCR assay utilising degenerate primers targeting a 250 bp region of the polymerase gene (VanDevanter et al.,

1996). To test for mycoplasma in conjunctival and oral swab samples of *C. porteri* and *C. vanderburghi* tortoises ($n = 278$), we adapted a qPCR protocol for use in a conventional PCR. This protocol targets the intergenic region between the 16S and 23S genes (Rebelo et al., 2011). Any PCR products were resolved in 1.5% agarose gels.

After screening oral swabs from a randomly selected group ($n = 90$) of *C. porteri* to test for Frog Virus 3 (FV3)-like ranaviruses, we used custom-made primers that amplify a 250 bp region of the major capsid protein gene (ranaV-F 5′-TTACATCCTCAACGCCTGGT-3′; ranaV-R 5′-GAGATCGCTGGTGTTGCCTA-3′) in a SYBR Green-based qPCR assay. These primers were designed by Primer3 and were tested in silico by the alignment with all FV3 and FV3-like ranavirus sequences available on Genbank. Cycling conditions were 95°C for 3 s, 40 cycles at 95°C for 15 s and 65°C for 30 s, followed by melt curve analysis. In order to validate the test, a control of FV3 ranavirus was amplified and sequenced. We tested mycoplasma for all western Santa Cruz and Alcedo Volcano tortoises as part of the broader health study conducted for these species. We selected a smaller number of samples to be tested for ranavirus due to the inability to conduct qPCR in Galapagos and limited funds available; however, no mortality or morbidity events have been reported in Galapagos frogs or reptiles that might be compatible with ranavirus. For the remaining species that had been opportunistically sampled we prioritised testing for adenovirus and herpesvirus as they had been previously detected in *C. porteri* and *C. vandenburghi*. In western Santa Cruz, where tortoises were sampled during humid and dry seasons, we used a Pearson's chi-square test to assess the influence of season in viral prevalence, as described by Kane et al. (2017).

2.4 | Sequencing and phylogenetic analyses

We subjected all PCR positive samples to Sanger sequencing. We considered a sample truly positive if a clear sequence was obtained. Nucleotide and deduced amino acid sequences were subjected to BLAST searches. Adenovirus sequences were aligned with 18 other adenovirus sequences found in GenBank. Herpesvirus sequences were aligned with 23 other herpesvirus sequences. Because there were gaps that made translation into amino acids problematic, we aligned the deduced amino acid sequences and subjected them to a protein BLAST search. In both groups, we calculated p-distances for nucleotide and deduced amino acid sequences. We inferred phylogenetic trees from nucleotide and deduced amino acid alignments using a maximum likelihood algorithm with a bootstrap frequency of 1000 replications (Mega 7.0). To assess for differences of pathogen prevalence between tortoise species, we used a Pearson's chi-square test ($p < .05$) and observed the adjusted standardised residuals.

3 | RESULTS

We obtained a total of 31 clean sequences 209 bp in length from adenovirus qPCR. Out of all sequences, we obtained two consistently different nucleotide sequence types (AdVntST-1 and AdVntST-2). These sequence data have been submitted to the EMBL databases under accession numbers OU508386 and OU508387, respectively. The two nucleotide sequences translated into two amino acid sequences (AdVaaST-1 and AdVaaST-2). The identities between AdVntST-1 and AdVntST-2 and between AdVaaST-1 and AdVaaST-2 were 95.6% and 91.2%, respectively. Based on alignments of the DNA polymerase gene and through maximum likelihood phylogenetic analyses, we found both novel adenoviruses to be most similar to red footed tortoise adenovirus 2 by nucleotide sequence (identities of 79.5% and 77.0%, respectively), and red footed tortoise adenovirus 1 based on amino acid sequence (79.4%). AdVaaST-1 and AdVaaST-2 form a clade with a bootstrap value of 0.96 (Figure 1), lending support to the placement of these viruses as novel species; therefore, we hereafter refer to them as *Chelonoidis adenovirus 1* (CheAdV1) and *Chelonoidis adenovirus 2* (CheAdV2). Clustered by tortoise species, 23 out of 208 tortoises (11.1%; 95% CI 6.8–15.3) tested positive for adenovirus in western Santa Cruz (*C. porteri*), 1 of 55 tortoises (1.8%; 95% CI 0.0–5.35) in eastern Santa Cruz (*C. donfaustoi*) and 7 of 78 (9%; 95% CI 2.63–15.32) in Alcedo (*C. vandenburghi*). No tortoises tested positive from Wolf Volcano, Española Island and the captive breeding facilities. In Table 1, we report the nucleotide and amino acid sequences obtained for the different tortoise species. Statistical differences were observed in adenovirus prevalence (chi-squared, $p < .05$), with *C. porteri* presenting a higher prevalence (11.1%) than expected (7%) and no detection in *C. becki* (0%). *C. donfaustoi* also presented a low prevalence (1.8%), but statistics were not significant. In Santa Cruz, all tortoises that were positive for adenovirus presented CheAdV1 except for one individual. The one tortoise from western Santa Cruz that presented the CheAdV2 sequence was an adult female tortoise tagged in 2019 as part of the long-term movement ecology research conducted by the GTMEP. In contrast, 6 out of 7 adenovirus-positive tortoises from Alcedo Volcano presented CheAdV2, and only one individual tested positive for CheAdV1. One of the tortoises that had tested positive for CheAdV2 was an adult female that had been monitored with a GPS tag since 2010.

A total of twelve animals in this study tested positive for herpesviruses based on conventional PCR. Only one of the positives was from a conjunctival swab, while the other eleven positive results were from oral swabs. We obtained four 180 bp nucleotide sequences of herpesviruses (HVntST-1, HVntST-2, HVntST-3 and HVntST-4). These sequence data have been submitted to the EMBL databases under accession numbers OU508388, OU508389, OU508390 and OU508391, respectively). These four nucleotide sequences translated into two different amino acid sequences (HVaaST-1 and HVaaST-2). Sequence similarity between the three first ntSTs ranged from 96.9% to 99.4%, whereas the similarity between these three and HVntST-4 was lower with a range of 62.5–63.7%. HVntST-1 to HVntST-3 all translated the same deduced amino acid sequence (HVaaST-1); therefore, they could be considered the same viral species. HVaaST-1 was 64.2% similar to the deduced amino acid sequence from nucleotide sequence HVntST-4 (HVaaST-2). The highest score by BLAST of HVntST-1-2-3 was with Emydid herpesvirus 1 and Terrapene herpesvirus 2, with 69.2–71.1% and 68.6–69.8% similarity, respectively. The highest score

TABLE 1 Prevalence, nucleotide (ntST) and amino acid (aaST) sequences of adenoviruses (AdV) and herpesviruses (HV) clustered by Galapagos tortoise location and species

Island	Species	Common name	Free/captive	AdV ntST	AdV aaST	Positive samples AdV	Prevalence AdV (95% CI)	HV ntST	HV aaST	Positive samples HV	Prevalence HV (95% CI)
Santa Cruz	*C. porteri*	Western	Free-living	ntST1	aaST1	22/208	10.58%	ntST1	aaST1	6/208	2.88%
		Santa Cruz					(6.40- 14.76)				(0.61–5.16)
				ntST2	aaST2	1/208	0.48%	-	-	-	-
							(0.00- 1.42)				
	C. donfaustoi	Eastern	Free-living	ntST1	aaST1	1/55	1.82%	-	-	0/55	-
		Santa Cruz					(0.00–5.35)				
Isabela	*C. vandenburghi*	Alcedo	Free-living	ntST 1	aaST1	1/78	1.28%	ntST1	aaST1	1/78	1.3%
		Volcano					(0.00–3.78)				(0.0–3.78)
				ntST2	aaST2	6/78	7.69%	-	-	-	-
							(1.78–13.61)				
	C. becki	Wolf Volcano	Free-living	-	–	0/55	–	ntST3	aaST1	1/55	1.82% (0.00–5.35)
				-	-	-	-	ntST4	aaST2	1/55	1.82% (0.00–5.35)
Española	*C. hoodensis*	Española	Free-living	-	-	0/45	-	-	-	0/45	-
			Captive	-	-	0/16	-	-	-	0/16	-
Hibrids	*C. becki hybrids*	Wolf Hybrids	*Captive*	-	-	0/5	-	ntST2	aaST1	3/5	60% (17.1–100.0)

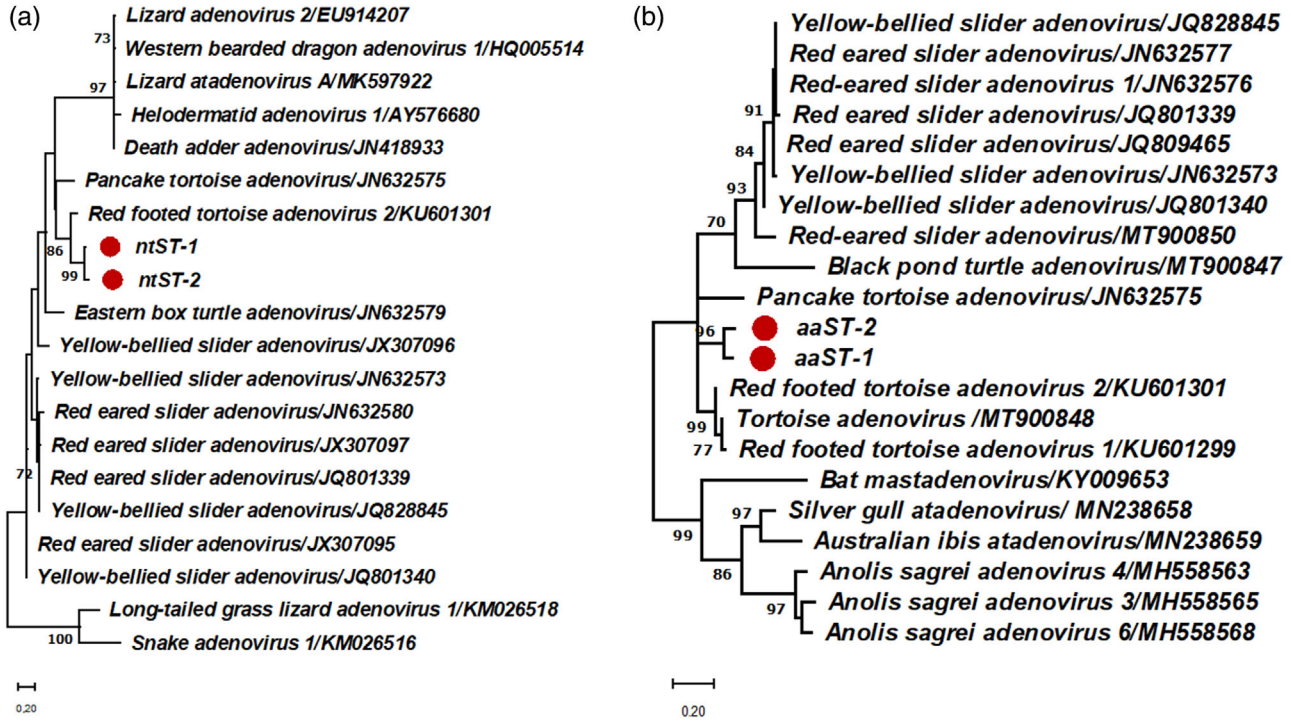

FIGURE 1 Maximum likelihood phylogenetic trees with 1000 bootstrap replications of nucleotide (a) and deduced amino acid (b) of 20 and 21 adenoviral DNA-dependent DNA polymerase gene sequences, respectively. The names correspond to Genbank name plus the GenBank accession number. The numbers near the branches represent the bootstrap frequency. All values under 70 have been omitted. Red dots denote the novel sequences described for Galapagos giant tortoises in the present study (EMBL accession numbers OU508386 and OU508387, respectively).

obtained by BLAST of HVaaST-1 was with the *Emydoidea blandingii* herpesvirus 2 (81.1%). However, HVntST-4 was highly divergent from any sequence previously detected, with a *Bubo bubo* herpesvirus as the most similar sequence based on nucleotide sequence (66.9%), while HVaaST-2 had an identity of 66% with Loggerhead (*Caretta caretta*) orocutaneous herpesvirus. HVntST-1 to HVntST-3 formed a clade that fell as a sister group to Terrapene herpesvirus 2, with a bootstrap value of 0.86 (Figure 2a). HVntST-4 and the corresponding translated HVaaST-2 did not group with the other three novel sequences nor with other herpesviruses detected in tortoises (Figure 2). The genetic distance seen between these and other characterised herpesviruses is consistent with placement of these viruses as novel species, and therefore we refer to them as *Chelonoidis herpesvirus 1* (CheHV1) and *Chelonoidis herpesvirus 2* (CheHV2). CheHV1 was detected in Santa Cruz and Isabela (Alcedo and Wolf Volcanoes) Islands, as well as in captive tortoises maintained at the Don Fausto Llerena Breeding Center in Santa Cruz. By contrast, CheHV2 was only found in a free-living adult male tortoise from Wolf Volcano. Clustered by tortoise species, 6 out of 208 tortoises (2.88%; 95% CI 0.61–5.16) were positive in western Santa Cruz (*C. porteri*), 1 of 78 (1.3%; 95% CI 0.0–3.78) in Alcedo (*C. vanderburghi*) and 2 of 55 (3.6%; 95% CI 0.0–8.58) in Wolf (*C. becki*) (Table 1). Interestingly, we had no herpesvirus positive tortoises from the eastern Santa Cruz ($n = 55$) or Española Island ($n = 45$) populations. No significant differences were observed in HV prevalence between free-living animals of the five tortoise species. In Figure 3, we represent the prevalences of free-living individuals for CheAdV1 and CheAdV2, and CheHV1 and CheHV2 clustered by species.

Of the tortoises maintained in captivity at the GNP breeding centre, three out of five sharing the same enclosure tested positive for HV (*C. becki* hybrids) (60%; 95% CI 17.1–100.0), including one adult female tortoise that presented with mucoid/diphtheric stomatitis, rhinitis, glossitis, conjunctivitis, blepharedema, mucopurulent nasal discharge, severe lethargy, cloacal ulcers, incoordination and torticollis on 29 August 2018. This animal was treated by the GNP veterinary service for up to 3 weeks (ceftazidime 20 mg/kg every 72 h, meloxicam 0.2 mg/kg, vitamin B and Fe) until it recovered. However, after 2 years, some physical damage still persists, including severe torticollis. Whether these symptoms were associated with the presence of CheHV1 remains unknown. No captive tortoises from Española Island tested positive for herpesvirus.

All free-living animals included in the current study were in apparently good condition based on physical exam performed during the sample collection (BCI based on weight and morphometrics and no visual signs of disease, discharge, lethargy or emaciation). No animals were recaptured or monitored over time other than those tagged with GPS devices. We did not have any individual that presented coinfection of both herpesvirus and adenovirus. All tortoises tested were negative for FV3 ranavirus ($n = 90$) and mycoplasma ($n = 278$) based on qPCR.

In western Santa Cruz, where tortoises were sampled during dry and humid seasons, more individuals tested positive for HV and AV during

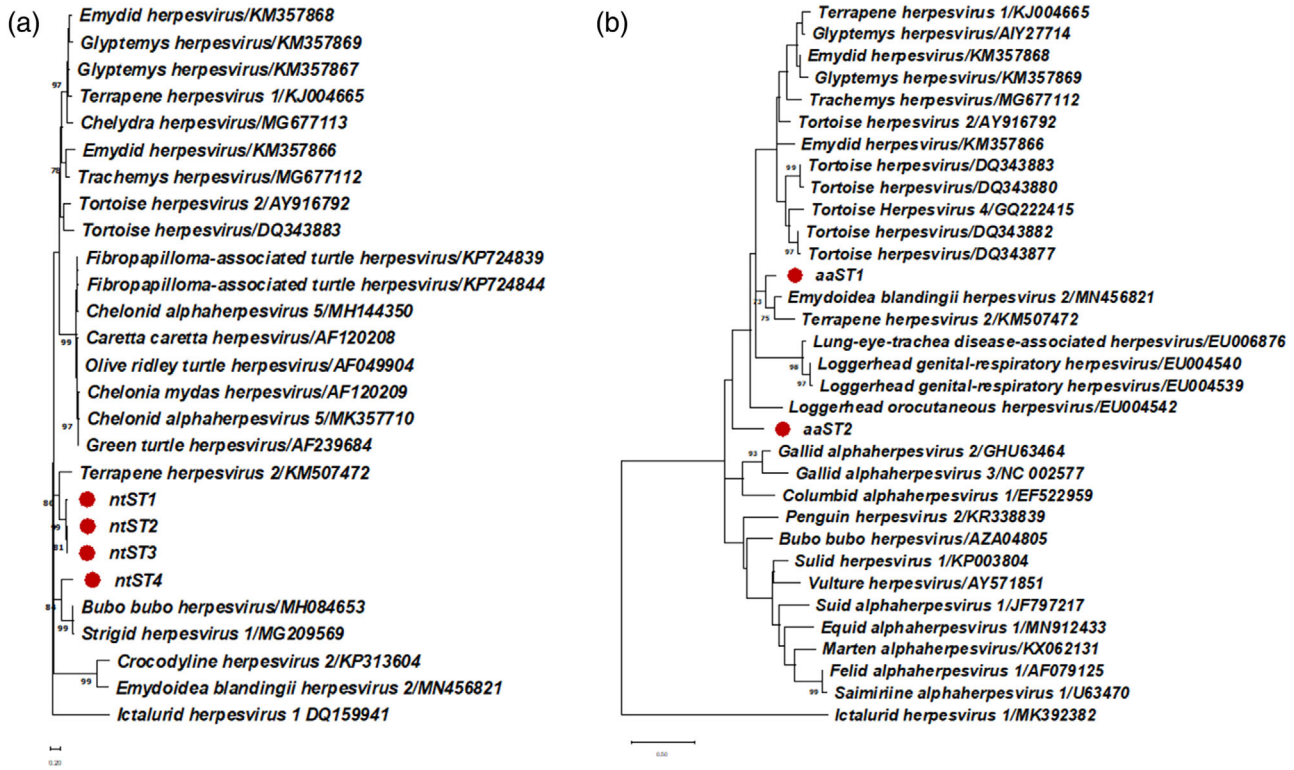

FIGURE 2 Maximum likelihood phylogenetic trees with 1000 bootstrap replications of nucleotide (a) and deduced amino acid (b) of 27 and 33 herpesviral DNA-dependent DNA polymerase gene sequences, respectively. The names correspond to Genbank name plus the GenBank accession number. The numbers near the branches represent the bootstrap frequency. All values under 70 have been omitted. Red dots denote the sequences described for Galapagos giant tortoises in the present study (EMBL accession numbers OU508388, OU508389, OU508390 and OU508391, respectively). Ictalurid herpesvirus 1 is included as an outgroup.

the humid season; however, statistics were not significant (AV $p = .25$ and HV $p = .09$).

4 | DISCUSSION

In the course of health assessments and exams on several species of giant tortoises in the Galapagos Islands, we discovered two novel viral sequences of herpesviruses and two of adenoviruses, which we describe here. Interestingly, we found a range of prevalences among species and locations, with western Santa Cruz (*C. porteri*) and Alcedo tortoises (*C. vandenburghi*) being the only two species in which herpesvirus and adenovirus were both documented within the same population. The prevalence of adenovirus was higher in *C. porteri* (11.1%), the population of tortoises most exposed to anthropogenic activities as indicated by the presence of antimicrobial resistance genes and other human impacts (Nieto-Claudin et al., 2021). Thirty-nine per cent of the samples that had tested positive for CheAdV in *C. porteri* were found in tortoises sampled within cattle farms, and another 39% of positive individuals were sampled within touristic farms or private properties in the highlands of Santa Cruz, used for ecotourism. Only 22% of the tortoises that had tested positive in Santa Cruz were sampled in protected areas of the National Park. By contrast, in the eastern Santa Cruz tortoise species, a species restricted to a small area of Santa Cruz and with much less interaction with human activities, only one individual of 55 tested was positive for CheAdV1 (1.8%) in an agricultural area, and no herpesvirus was detected. Considering that tortoises were translocated by the Galapagos National Park Services between the East and West tortoise populations of Santa Cruz in recent years, and prior to the designation of *C. donfaustoi* as a separate species in 2015, we cannot refute the possibility that adenovirus infection of eastern Santa Cruz tortoises may have been anthropogenically introduced in association with previous management practices.

In Alcedo Volcano, there was a relatively high prevalence of adenovirus (9%) and a low prevalence of herpesvirus (1.3%). We found both viruses within *C. porteri* and *C. vandenburghi* populations, which may be due to how closely related these species are to one another. Western Santa Cruz tortoises (*C. porteri*) are more related to Alcedo tortoise species (*C. vandenburghi*) than to the eastern Santa Cruz species (*C. donfaustoi*), which is more closely related to San Cristóbal tortoises (*C. chathamensis*) (Poulakakis et al., 2020). Interestingly, the isolation between the western Santa Cruz and the Alcedo species may have resulted in the coevolution of tortoises and their viruses, as the most frequent sequence found in *C. porteri* was CheAdV1 (only 1 out of 23 individuals tested positive for CheAdV2), whereas in *C. vandenburghi* 6 out of 7 positive tortoises carried CheAdV2.

While we did not detect adenovirus in Wolf Volcano species, we did detect two different species of herpesvirus in this population, with

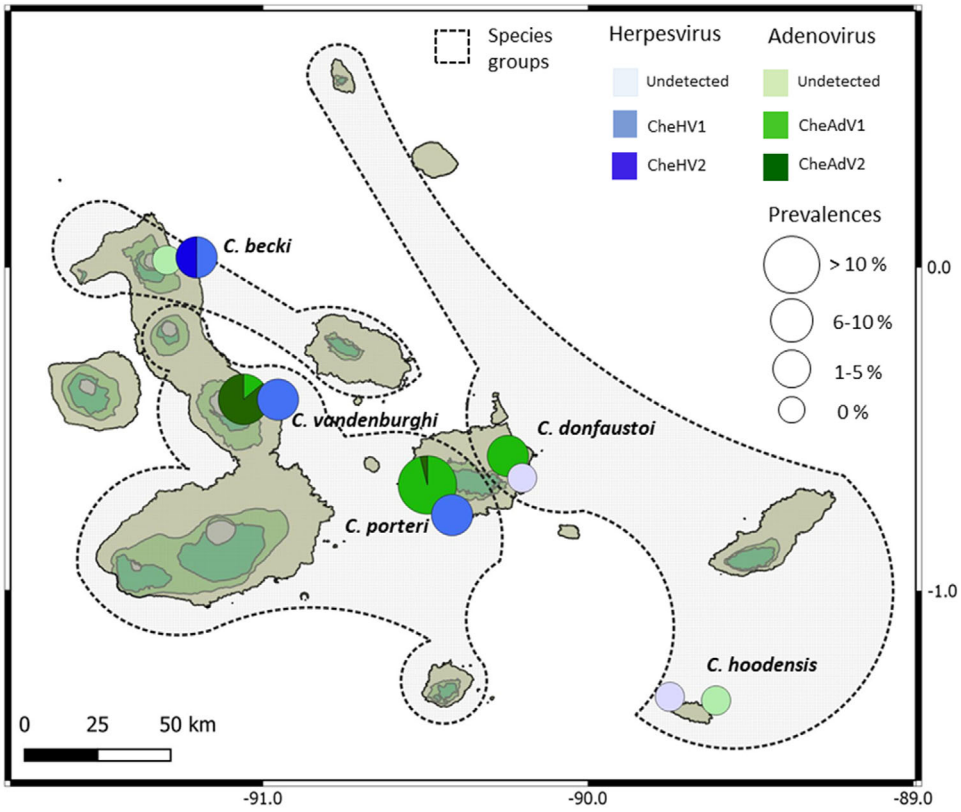

FIGURE 3 Graphical representation of CheAdV1 and CheAdV2 and CheHV1 and CheHV2 for Galapagos giant tortoises clustered by species and according to the three main phylogenetic species groups described by Poulakakis et al. (2020)

CheHV2 detected in one adult male. This information could be of high conservation concern since Wolf tortoises coexist with the unique and endemic pink land-iguana (*Conolophus marthae*), as well as with yellow iguanas (*Conolophus subcristatus*). Based on phylogenetics, the species most related to Wolf Volcano tortoises is *C. darwini* from Santiago Island. Studying the presence and prevalence of herpesvirus in Santiago could therefore provide more information about the potential evolution of these viruses within Galapagos tortoise species. Moreover, hybrids of the extinct Floreana and Pinta tortoises have been found in Wolf, suggesting that this population was historically highly influenced by human interventions, with pirates, whalers and researchers moving tortoises between islands during the 19th and 20th centuries (Garrick et al., 2012; Tapia, 2020). These movements also may have influenced the presence of certain pathogens such as herpesviruses.

Viruses that have evolved with their host generally do not pose a high risk of infection or morbidity unless the virus jumps host species or mutates into a virulent form (Mandl et al., 2015). However, cataloging existing viruses is important, since a virus that might be endemic in one species could be virulent in another (Rosenberg, 2015). Even well-adapted pathogens may result in a slight cost in terms of immunity, which might be exacerbated if external or additional factors coexist (e.g. stress, malnutrition, aging, comorbidity). Captive-breeding programs and wildlife translocation, while successful to prevent extinctions under some circumstances, may also facilitate the spread of microorganisms and disease if animals are translocated without comprehensive risk analyses and proper veterinary advice (Deem, 2012; Hunter et al., 2019; Kock et al., 2010; Sainsbury & Vaughan-Higgins, 2012). In this sense, the tortoise restoration initiatives carried out across the archipelago could pose the risk of pathogen translocation if no diagnostics are performed. In Galapagos, where several species of endemic and unique reptiles coexist, describing existing viruses of all reptile species will provide a valuable tool for management decisions involving translocation of individuals and species.

In 2019, one juvenile tortoise maintained in captivity by the Galapagos National Park Service of Santa Cruz presented respiratory signs (blepharitis, conjunctivitis, ocular and nasal discharge) and tested positive for CheAdV2. It is possible that these clinical signs are associated with adenovirus and/or other tortoise pathogens. Considering that this individual was maintained in a high-density enclosure with another 25 tortoises from different species, all of them in poor health conditions, and most of them also PCR positive to CheAV, we cannot rule out that adenovirus may have played a role in the observed signs. Additionally, the evidence of a captive tortoise positive for CheHV1 with severe symptoms suggests that herpesvirus also could be pathogenic under specific conditions, such as with immunosuppression due to stress, captivity or transportation. The determination of these clinical signs being due to CheAV or CheHV1 infection in tortoises warrant further investigation. Regardless, these findings support the importance of a systematic wildlife health surveillance within the archipelago for both captive and free-living animals, to identify current and potential new

threats that may compromise wildlife health and well-being and support the rapid detection of novel pathogens that may be introduced to the archipelago.

Based on our results, we obtained more positive herpesvirus diagnoses from oral swabs than from ocular swabs in Galapagos tortoises; therefore, we suggest standardising the methodology to conduct long term tortoise surveillance of herpesvirus and adenovirus based on oral and cloacal swabs, respectively, and for using either PCR or qPCR protocols. We suggest the use of both adenovirus consensus primers and the specific primers described within this work when working with Galapagos tortoise samples, as specific primers may produce cleaner sequences and less false positive results than consensus primers.

Tortoises from western Santa Cruz and Alcedo Volcano included in the current study were also included in a research project testing faecal samples for antimicrobial-resistant genes (ARGs). Five tortoises that had tested positive for CheAdV1 (21.7%) and one that tested positive for CheHV1 (16.7%) in Santa Cruz corresponded to the same location where a hotspot of antimicrobial resistance was also described (Nieto-Claudin et al., 2021). This particular location is a cattle farm in the highlands of Santa Cruz. The average number of ARGs on the microbiome of the six individuals that tested positive within this farm was 7.3, higher than the prevalence observed for this population of giant tortoises (4 genes per sample based on Nieto-Claudin et al., 2021; $p = .001$, CI = 95%). This suggests a potential correlation between the presence of viruses and the prevalence of ARGs observed in western Santa Cruz. Exposure to antibiotics could disrupt the normal commensal flora of wildlife, and the presence of bacteria carrying anthropogenically associated ARGs may indicate the possibility for zoonotic disease transmission between humans and wildlife (Vittecoq et al., 2016). Some authors have also suggested that microbial dysbiosis can result in enhanced virulence of colonising pathogens (Thomason et al., 2017) with critical implications for the health of wildlife, domestic animals and humans. While it cannot be assumed that CheAV and/or CheHV are pathogenic for giant tortoises without further studies, the potential association of ARGs and tortoise virus presence, while not an outcome from the present work, deserves further investigation. It is possible that anthropogenic-induced changes in microbiota resulting in the presence of resistant bacteria can effect changes in the performance of the host immune system and possibly lead to more viral shedding.

Based on the different strains and prevalences of herpesvirus and adenovirus observed among tortoise species, we suggest expanding tortoise health monitoring into Pinzón, Santiago, San Cristóbal and other locations within Isabela Island, as well as including other reptile species in close contact with free-living tortoises (e.g., land iguanas) to better understand the presence and prevalence of these viruses across the archipelago. Given the evolutionary relationship between species, CheHV1 and CheHV2 could be found in tortoise species closely related to Wolf Volcano such as Santiago tortoises, whereas CheAdV1 and CheAdV2 could be found in species closely related to Alcedo and western Santa Cruz, such as *C. vicina* and *C. microphyes* (Isabela Island). Continued screening is crucial to determine if these viruses play a role in tortoise fitness, morbidity and survival. This information will allow us to provide proper recommendations to the Galapagos National Park Directorate and other institutions to improve the management and reintroduction plans for these unique species, which would include strategies to prohibit the movement of potential pathogens across islands and populations not previously exposed to these viruses.

CONFLICT OF INTEREST

Ainoa Nieto Claudin, Fernando Esperón, Kathleen Apakupakul, Irene Peña and Sharon L. Deem declare there is no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the Galapagos National Park Directorate, Saint Louis Zoo Institute for Conservation Medicine, Saint Louis Wild-Care Institute Center for Chelonian Conservation, the AAZV Wild Animal Health Fund (WAHF #37), Houston Zoo, Galapagos Conservation Trust, Charles Darwin Foundation, Ecoventura, Galapagos Conservancy and Linda Esler. A special recognition for their contributions goes to Stephen Blake, Freddy Cabrera, José Haro, Anne Guezou, Andrea Loyola, Washington Tapia, Carolina Carrión, Karina Ramón, Laura Kleinschmidt, Surya Castillo, Jamie Palmer, Cristina Chasipanta, Elena Neves, Irene Sacristán, Encarnación Madueño, Santiago Cano and Olga Calatayud. This publication is contribution number 2425 of the Charles Darwin Foundation for the Galapagos Islands.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. We followed the guidelines of the Galapagos National Park Directorate and the International Animal Care and Use Committee from GREFA (Spain) with registration number 17/001.

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REFERENCES

Arteaga, A., & Guayasamin, J. M. (2020). Chelonoidis becki. In A. Arteaga, L. Bustamante, J. Vieira, & J. M. Guayasamin (Eds.), *Reptiles of Ecuador: Life in the middle of the world*. Available from: Quito: Universidad Tecnológica Indoamericana. Www.tropicalherpi ng.com

Agius, J. E., Phalen, D. N., Rose, K., & Eden, J. S. (2019). New insights into Sauropsid Papillomaviridae evolution and epizootiology: Discovery of two novel papillomaviruses in native and invasive Island geckos. *Virus Evolution*, *5*, 1–12.

Benitez-Capistros, F., Couenberg, P., Nieto, A., Cabrera, F., & Blake, S. (2019). Identifying shared strategies and solutions to the human-giant tortoise interactions in Santa Cruz, Galapagos: A nominal group technique application. *Sustain*, *11*, 1–25.

Blake, S., Wikelski, M., Cabrera, F., Guezou, A., Silva, M., Sadeghayobi, E., Yackulic, C. B., & Jaramillo, P. (2012). Seed dispersal by Galápagos tortoises. *Journal of Biogeography*, *39*, 1961–1972.

Blake, S., Yackulic, C. B., Wikelski, M., Gibbs, J. P., Deem, S., & Villamar, F. (2015). La migración de las tortugas gigantes de Galápagos requiere de

esfuerzos de conservación a escala de paisaje. In *En: Informe Galápagos 2013-2014. DPNG, CGREG, FCD y GC. Puerto Ayora, Galápagos, Ecuador.* (pp. 144–150).

Blake, S., Guézou, A., Deem, S. L., Yackulic, C. B., & Cabrera, F. (2015). The Dominance of Introduced Plant Species in the Diets of Migratory Galapagos Tortoises Increases with Elevation on a Human-Occupied Island. *Biotropica*, *47*(2), 246–258. https://doi.org/10.1111/btp.12195

Cayot, L. J., Gibbs, J. P., Tapia, W., & Caccone, A. (2017a). Chelonoidis porteri. The IUCN Red List of Threatened Species 2017: E.T9026A82777132. https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T9026A82777132.en Downloaded on 21 June 2021.

Cayot, L. J., Gibbs, J. P., Tapia, W., & Caccone, A. (2017b). Chelonoidis hoodensis. The IUCN Red List of Threatened Species 2017: E.T9024A82777079. https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T9024A82777079.en Downloaded on 24 March 2021.

Chinchar, V. G. (2002). Ranaviruses (family Iridoviridae): Emerging cold-blooded killers Brief Review. *Archives of Virology*, *147*, 447–470.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife—Threats to biodiversity and human health. Philosophical Transactions of the Royal Society B: Biological Sciences, *287*, 443–450.

Deem, S. L. (2012). Fowler's zoo and wild animal medicine: Current therapy 7. In R. E. Miller & M. E. Fowler (Eds.), *Disease risk analysis in wildlife health studies*. Saunders Elsevier. https://doi.org/10.1016/B978-1-4377-1986-4.00001-9

Doszpoly, A., Wellehan, J. F. X., Childress, A. L., Tarján, Z. L., & Kovács, E. R. (2013). Partial characterization of a new adenovirus lineage discovered in testudinoid turtles. Infection, Genetics and Evolution, *17*, 106–112.

Fèvre, E. M., Bronsvoort, B., Hamilton, K. A., & Cleaveland, S. (2006). Animal movements and the spread of infectious diseases. *Trends in Microbiology*, *14*, 125–131.

Garrick, R. C., Benavides, E., Russello, M. A., Gibbs, J. P., Poulakakis, N., Dion, K. B., Hyseni, C., Kajdacsi, B., Márquez, L., Bahan, S., Ciofi, C., Tapia, W., & Caccone, A. (2012). Genetic rediscovery of an "extinct" Galápagos giant tortoise species. *Current Biology*, *22*, R10–R11.

Gibbons, P. M., & Steffes, Z. J. (2013). Emerging infectious diseases of chelonians. *Veterinary Clinics of North America: Exotic Animal Practice*, *16*, 303–317.

Gibbs, J. P., Hunter, E. A., Shoemaker, K. T., Tapia, W. H., & Cayot, L. J. (2014). Demographic Outcomes and Ecosystem Implications of Giant Tortoise Reintroduction to Española Island, Galapagos. *Plos One*, *9*, E110742.

Hunter, E. A., Gibbs, J. P., Cayot, L. J., Tapia, W., Quinzin, M. C., Miller, J. M., Caccone, A., & Shoemaker, K. T. (2019). Seeking compromise across competing goals in conservation translocations: The case of the 'extinct' Floreana Island Galapagos giant tortoise. *Journal of Applied Ecology*, *57*, 136–148.

IUCN (2020). The IUCN Red List of Threatened Species. Version 2020–3. https://www.iucnredlist.org (Accessed 14 June 2021).

Jacobson, E. R., Brown, M. B., Wendland, L. D., Brown, D. R., Klein, P. A., Christopher, M. M., & Berry, K. H. (2014). Mycoplasmosis and upper respiratory tract disease of tortoises: A review and update. *Veterinary Journal*, *201*, 257–264.

Kane, L. P., Allender, M. C., Archer, G., Dzhaman, E., Pauley, J., Moore, R., Ruiz, M. O., Smith, R. L., Byrd, J., & Phillips, C. A. (2017). Prevalence of Terrapene herpesvirus 1 in free-ranging eastern box turtles (*Terrapene carolina carolina*) in Tennessee and Illinois. Journal of Wildlife Diseases,*53*, 1–11.

Kock, R. A., Woodford, M. H., & Rossiter, P. B. (2010). Disease risks associated with the translocation of wildlife. *OIE Revue Scientifique et Technique*, *29*, 329–350.

Kolesnik, E., Obiegala, A., & Marschang, R. E. (2017). Detection of *Mycoplasma* spp., herpesviruses, topiviruses, and ferlaviruses in samples from chelonians in Europe. *Journal of Veterinary Diagnostic Investigation*, *29*, 820–832.

Lavoie, C., Cruz, F., Carrion, G. V., Campbell, K., Donlan, C. J., Harcourt, S., & Moya, M. (2007). *The thematic atlas of project Isabela: An illustrative document describing, step-by-step, the biggest successful goat eradication project on the Galapagos Islands, 1998–2006*. Puerto Ayora, Galapagos: Charles Darwin Foundation.

Lwanga, S. K., & Lemeshow, S. (1991). Sample size determination in health studies: A practical manual. World Health Organization.

Mandl, J. N., Ahmed, R., Barreiro, L. B., Daszak, P., Epstein, J. H., Virgin, H. W., & Feinberg, M. B. (2015). Reservoir host immune responses to emerging zoonotic viruses. *Cell*, *160*, 20–35.

Marschang, R. E. (2011). Viruses infecting reptiles. *Viruses*, *3*, 2087–2126.

Marschang, R. E. (2019). Virology. In S. J. S., Stephen & J., Divers (Eds.), *Mader's reptile and amphibian medicine and surgery* (3rd edn., pp. 247–269). Elsevier. https://doi.org/10.1016/B978-0-323-48253-0.00030-1

Marschang, R. E., Bergmann, S. M., & Miller, D. L. (2008). Ranavirus infection in amphibians. *EAZWV Transmissible Disease Fact Sheet Sheet* 1–3.

Massei, G., Quy, R. J., Gurney, J., & Cowan, D. P. (2010). Can translocations be used to mitigate humanwildlife conflicts? *Wildlife Research*, *37*, 428–439.

Nieto-Claudin, A., Deem, S. L., Rodriguez, C., Cano, S., Moity, N., Cabrera, F., & Esperón, F. (2021a). Antimicrobial resistance in Galapagos tortoises as an indicator of the growing human footprint. *Environmental Pollution*, *284*, 117453. https://doi.org/10.1016/j.envpol.2021.117453.

Nieto-Claudin, A., Palmer, J. L., Esperón, F., & Deem, S. L. (2021b). Haematology and plasma biochemistry reference intervals for the critically endangered western Santa Cruz Galapagos tortoise (Chelonoidis porteri). *Conserv Physiol*, *9*(1): coab019; https://doi.org/10.1093/conphys/coab019.

Origgi, F. C. (2012). Testudinid herpesviruses: A review. *Journal of Herpetological Medicine and Surgery*, *22*, 42–54.

Palmer, J. L., Blake, S., Wellehan, J. F. X., Childress, A. L., & Deem, S. L. (2016). Clinical *Mycoplasma* sp. infections in free-living three-toed box turtles (*Terrapene carolina* triunguis) in Missouri, USA. *Journal of Wildlife Diseases*, *52*, 378–382.

Poulakakis, N., Miller, J. M., Jensen, E. L., Beheregaray, L. B., Russello, M. A., Glaberman, S., Boore, J., & Caccone, A. (2020). Colonization history of Galapagos giant tortoises: Insights from mitogenomes support the progression rule. *Journal of Zoological Systematics and Evolutionary Research*, *58*, 1262–1275.

Rebelo, A. R., Parker, L., & Cai, H. Y. (2011). Use of high-resolution melting curve analysis to identify Mycoplasma species commonly isolated from ruminant, avian, and canine samples. *Journal of Veterinary Diagnostic Investigation*, *23*, 932–936.

Rhodin, A. G. J., Stanford, C. B., Van, D. P. P., Eisemberg, C., Luiselli, L., Mittermeier, R. A., Hudson, R., Horne, B. D., Goode, E. V., Kuchling, G., Walde, A., Baard, E. H. W., Berry, K. H., Bertolero, A., Blanck, T. E. G., Bour, R., Buhlmann, K. A., Cayot, L. J.,… Vogt, R. C. (2018). Global conservation status of turtles and tortoises (order testudines). *Chelonian Conservation and Biology*, *17*, 135.

Rivera, S., Wellehan, J. F. X., McManamon, R., Innis, C. J., Garner, M. M., Raphael, B. L., Gregory, C. R., Latimer, K. S., Rodriguez, C. E., Diaz-Figueroa, O., Marlar, A. B., Nyaoke, A., Gates, A. E., Gilbert, K., Childress, A. L., Risatti, G. R., & Frasca, S. Jr. (2009). Systemic adenovirus infection in Sulawesi tortoises (Indotestudo forsteni) caused by a novel siadenovirus. *Journal of Veterinary Diagnostic Investigation*, *21*, 415–426.

Rosenberg, R. (2015). Detecting the emergence of novel, zoonotic viruses pathogenic to humans. *Cellular and Molecular Life Sciences*, *72*, 1115–1125.

Sainsbury, A. W., & Vaughan-Higgins, R. J. (2012). Analyzing disease risks associated with translocations. *Conservation Biology*, *26*, 442–452.

Sim, R. R., Allender, M. C., Crawford, L. K., Wack, A. N., Murphy, K. J., Mankowski, J. L., & Bronson, E. (2016). Ranavirus epizootic in captive Eastern Box Turtles (Terrapene carolina carolina) with concurrent herpesvirus and mycoplasma infection: Management and monitoring. *Journal of Zoo and Wildlife Medicine*, *47*, 256–270.

Sim, R. R., Norton, T. M., Bronson, E., Allender, M. C., Stedman, N., Childress, A. L., & Wellehan, J. F. X. (2015). Identification of a novel herpesvirus

in captive Eastern box turtles (Terrapene carolina carolina). *Veterinary Microbiology*, *175*, 218–223.

Stanford, C. B., Iverson, J. B., Rhodin, A. G. J., Van, D. P. P., Mittermeier, R. A., Kuchling, G., Berry, K. H., Bertolero, A., Bjorndal, K. A., Blanck, T. E. G., Buhlmann, K. A., Burke, R. L., Congdon, J. D., Diagne, T., Edwards, T., Eisemberg, C. C., Ennen, J. R., Forero-Medina, G., Frankel, M., …Walde, A. D., (2020). *Review turtles and tortoises are in trouble. Current Biology*, 30, R721–R735.

Tapia, W. (2018). Results from the first-ever census of the Eastern Santa Cruz Tortoise. Giant Tortoise Restoration Initiative. Available from: https://www.galapagos.org

Tapia, W. (2020). Breaking: Expedition to wolf locates tortoise with pinta genes. Giant Tortoise Restoration Initiative. Available from: https://www.galapagos.org

Tapia, W. (2021a). Española tortoises return home following closure of successful breeding program. Giant Tortoise Restoration Initiative. Available from: https://www.galapagos.org

Tapia, W. (2021b). Breaking: Giant tortoise population on alcedo deemed healthiest in galapagos. Giant Tortoise Restoration Initiative. Available from: https://www.galapagos.org

Thomason, C. A., Mullen, N., Belden, L. K., May, M., & Hawley, D. M. (2017). Resident microbiome disruption with antibiotics enhances virulence of a colonizing pathogen. *Scientific Reports*, *7*, 1–8.

VanDevanter, D. R., Warrener, P., Bennett, L., Schultz, E. R., Coulter, S., Garber, R. L., & Rose, T. M. (1996). Detection and analysis of diverse herpesviral species by consensus primer PCR. *Journal of Clinical Microbiology*, *34*, 1666–1671.

Vittecoq, M., Godreuil, S., Prugnolle, F., Durand, P., Brazier, L., Renaud, N., Arnal, A., Aberkane, S., Jean-Pierre, H., Gauthier-Clerc, M., Thomas, F., & Renaud, F. (2016). Antimicrobial resistance in wildlife. *Journal of Applied Ecology*, *53*, 519–529.

Wellehan, J. F. X., Johnson, A. J., Harrach, B., Benkö, M., Pessier, A. P., Johnson, C. M., Garner, M. M., Childress, A., & Jacobson, E. R. (2004). Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. *Journal of Virology*, *78*, 13366–13369.

Yonkers, S. B., Schneider, R., Reavill, D. R., Archer, L. L., Childress, A. L., & Wellehan, J. F. X. (2015). Coinfection with a novel fibropapilloma-associated herpesvirus and a novel *Spirorchis* sp. in an eastern box turtle (Terrapene carolina) in Florida. *Journal of Veterinary Diagnostic Investigation*, *27*, 408–413.

How to cite this article: Nieto-Claudin, A., Esperón, F., Apakupakul, K., Peña, I., & Deem, S. L. (2021). Health assessments uncover novel viral sequences in five species of Galapagos tortoises. *Transboundary and Emerging Diseases*, 1–11. https://doi.org/10.1111/tbed.14391