First detection of herpesvirus and frequency of mycoplasma infection in free-ranging Hermann's tortoises (*Testudo hermanni*), and in potential pet vectors

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Abstract

Two types of pathogens cause highly contagious upper respiratory tract diseases (URTD) in Chelonians: testudinid herpesviruses (TeHV) and a mycoplasma (*Mycoplasma agassizii*). In captivity, these infections are frequent and can provoke outbreaks. Pet trade generates international flow of tortoises, often without sanitary checking; individuals intentionally or accidentally released in the wild may spread pathogens. A better understanding of the transmission of infectious agents from captivity to wild tortoises is needed. Many exotic individuals have been introduced in populations of the endangered western Hermann's tortoise (*Testudo hermanni hermanni*), notably spur-thighed tortoises (*Testudo graeca*). We assessed the presence of TeHV and mycoplasma in native western Hermann's tortoises and in potential pet vectors in south-eastern France. Using a large sample (N=572 tortoises), this study revealed the worrying presence of herpesvirus in 7 free-ranging individuals (3 sub-populations). Additionally, *Mycoplasma agassizii* was detected in 15 of the 18 populations sampled with a frequency ranging from 3.4% (1 of 29 tortoises) to 25% (3 of 12 tortoises). Exotic spur-thighed tortoises showed high frequency of *Mycoplasma* infection in captivity (18.2%) and in individuals (50%) found in native Hermann's tortoise sub-populations, suggesting that this species could be a significant vector. The paucity of information of TeHV on European tortoise' URTD in natural settings, especially in combination with mycoplasma, prompts for further studies. Indeed, sick tortoises remain concealed and may not be easily detected in the field. Our results indicate that both the prevalence and health impact of URTD are high and should be scrutinized in the field.

Key words: Emerging infectious diseases (EIDs), *Mycoplasma agassizii*, Testudinidae, upper respiratory tract diseases (URTD), reptiles.

Introduction

Emerging infectious diseases (EIDs) represent a growing challenge for biodiversity conservation (Daszak et al., 2000; Deem et al., 2001). During the last decades, rapidly spreading diseases are suspected to have wreaked havoc worldwide among amphibians and reptiles (Daszak et al., 2000). In tortoises, *Testidinid herpesviruses* (TeHV) and *Mycoplasma* spp. are two dangerous pathogens for wild populations (Origgi, 2012; Marenzoni et al., 2018). Both are highly contagious and are involved in the Upper Respiratory Tract Disease Syndromes (URTD); they can provoke high morbidity and mortality (Brown et al., 1994; Goessling et al., 2019). For example, in the 80's, Mycoplasma epizooties were responsible of multiple collapses of desert tortoise populations in North America (Jacobson et al., 1991; Brown et al., 1994). Currently, several tortoise species such as Gopher tortoise (*Gopherus agassizii*), or captive spur-thighed tortoise (*Testudo graeca*) are impacted by URTD (Marschang and Schneider, 2007; Weitzman et al. 2017). Monitoring the health status of free-ranging chelonians, with a focus on URTD, is thus a conservation priority.

On the other hand, global trade of reptiles is flourishing; tens of thousands of individuals from an increasing number of species are displaced among continents under minimal (or non-existent for illegal trade) sanitary monitoring (Auliya et al., 2016). The resulting flows of individuals open major routes for the expansion of EIDs (DiGeronimo et al., 2019). Pet tortoises host both TeHV and *Mycoplasma* spp. (Martínez-Silvestre et al., 1999; Sandmeier et al., 2009; Lecis et al., 2011; Salinas et al., 2011; Origgi, 2012). The primary route of transmission of Herpesvirus and Mycoplasma is believed to be horizontal via contact between individuals (DiGeronimo et al., 2019). Interspecific transmission has been demonstrated (Origgi et al., 2004; Soares et al., 2004; Salinas et al., 2011). Tortoise species are often mixed up in cages or enclosures. Overall, very high prevalence of infection in captivity is expected (Kolesnik et al., 2017). Unfortunately, captive individuals are frequently

released into the wild, intentionally or accidentally, they can settle in novel habitats while carrying pathogens that may threaten native populations of tortoises (Sandmeier et al., 2009; Jacobson et al. 2014; Whitfield et al., 2018). This process opens highways for pathogens. The scarcity of investigations in natural populations, except in USA, means that possible role of pet tortoises as agents of contamination is not quantified in Europe, as in most parts of the world (Jacobson and Berry, 2012; Kane et al., 2017; Orton et al., 2020).

Fragile inbreed populations are particularly at risk. Reduced phenotypic diversity and genetic depression often hinder physiological and demographic resistances to diseases (Franckam et al. 2002; Spielman et al., 2004). Many species are subjected to all the threats above, including the not yet evaluated risk of simultaneous contamination by TeHV and Mycoplasma. The Hermann's tortoise provides a typical example of such a situation. Two Hermann tortoise subspecies are currently recognized (Pérez et al., 2013): the Western Hermann's tortoise (WHT, *Testudo hermanni hermanni*) that occurs west of the Po Valley in Italy (e.g. Italian Peninsula, Sardinia, Corsica, southeastern France, northeastern Spain) and the Eastern Hermann's tortoise *T. h. boettgeri* (EHT) found in Mediterranean regions of the Balkan Peninsula and in small islands spread in the eastern Mediterranean sea. The two subspecies come into contact in north eastern Italy where they possibly hybridize naturally (Pérez et al., 2013). The WTH is severely threatened by habitat loss and fragmentation, frequent fires, illegal harvesting and predation by feral animals. As a result, continental populations drastically decreased (Livoreil 2009). Previously abundant in continental southeastern France, relict isolated WHT sub-populations persist in the Massif des Maures (Var district, 83) and in adjacent plains (Livoreil, 2009; Bertolero et al., 2011). Delayed maturity, low fecundity and low population turn over mean that it is particularly sensitive to a decrease of adult survival (Bertolero et al., 2011).

Intensive and long-lasting legal and illegal pet trades (CITES 2014) provoked substantial introgression of EHT and of other tortoise species (e.g. spur-thighed tortoise) inside the natural repartition area of WTH (Martínez-Silvestre et al., 2001), while various species originating from other continents are sporadically found in the wild (unpublished data). These exotic tortoises easily breed in captivity are frequently owned as pets; they occur in many properties spread across the entire (remaining) distribution range of the native WHT. A considerable pool of captive individuals from various uncontrolled origins strongly enhances the likelihood of contacts with free-ranging native WHT.

Cross-species transmissions have been documented both for TeHV and mycoplasma; experiments demonstrated that these pathogens can infect Hermann and spur-thighed tortoises (Origgi et al*.,* 2004; Soares et al., 2004; Salinas et al., 2011). In Europe, TeHV-1 and -3 affect most of the Testudinidae species raised in captivity (Origgi, 2012; Marschang and Schneider, 2007). More generally, approximately48% of individuals belonging to various terrestrial and aquatic pet chelonians were positive for herpesvirus or mycoplasma while a positive correlation was observed between the two pathogen detection frequencies (Kolesnik et al., 2017). Like TeHV, mycoplasma was detected in tortoises kept in captivity and in outdoor enclosures in Europe (Lecis et al., 2011; Salinas et al., 2011).

Mortality due to TeHV and mycoplasma is well documented in wild tortoises in USA (Jacobson et al., 2012, 2014), but only in captivity in European tortoises (Soares, 2004; Kolesnik, et al., 2017). In the latter, mortality rate caused by TeHV is higher in Hermann's tortoise compared to other species, suggesting a recent and more deleterious contact between the host and the pathogen (Soares et al*.,* 2004). Possible occurrence and consequences of co-infection by TeHV and mycoplasma are not documented, at least in the Hermann's tortoise. Both frequency and possible severity of TeHV and mycoplasma infections remain unexplored in native populations of tortoises in Europe. Overall, possible

impact of worrying EIDs has not been assessed in natural setting in the Mediterranean basin that hosts many endemic tortoises (Gracià et al., 2020). This issue is urgent because exogenous tortoises are frequently observed in the wild where they may carry new pathogens (Lecis et al., 2011; Hidalgo-Vila et al., 2020).

Accurate monitoring of the frequency of TeHV and mycoplasma infections and information about the prevalence of URTD in the remaining populations of WHT is thus needed. This, notably because these pathogens can induce deleterious chronic diseases that are not easily diagnosed in long-lived organisms (Sandmeier et al., 2013). This study reports results from the first comprehensive survey across the distribution range of the WHT in continental France.

Material and methods

Tortoise sampling

From 2012 to 2016, 18 sites were monitored covering most of the distribution area of the WTH subspecies in continental France (besides these surveys, individuals were also opportunistically sampled throughout the distribution area) (Figure 1; Livoreil, 2009). Free-ranging tortoises are cryptic; thus, in addition to visual searching, trained dogs were used (Ballouard et al., 2019). Surveys were performed during the activity season of the species (from March to October), mostly in spring (111 searching days in spring, 34 in summer and 15 in autumn). All tortoises sighted were captured: 457 free-ranging individuals (421 WHT and 36 exotic specimens) were sampled (25.5 tortoises per site on average). In addition, 95 captive (pet) tortoises were sampled in 21 different properties in surrounding areas (5.2 individuals per property on average). Finally, 20 vagrant isolated individuals found in urban, or peri-urban areas, were also tested; likely they were pets intentionally released or that escaped from gardens. Most tortoises were adult (96%) and sex ratio was balanced (281

146 females, 267 males, 24 immatures). Overall, with respect to sampling context, we obtained
147 three categories of individuals: a) free-ranging, b) captive (pet), and c) vagrant (N total=572).
148 Each category contained both native and exotic tortoises.

149 Individuals were assigned to species (e.g. *T. graeca vs. T. hermanni*) or subspecies (WHT
150 *vs.* EHT) according to their morphological characteristics. *Testudo* species are easily
151 distinguished (Bertolero et al. 2011), subspecies not. The following criteria were used to
152 discriminate WHT from EHT: yellow subocular scales, black continuous plastral bands,
153 narrow vertebral scute, supracaudal scute divided, long corneous tip of the tail, corneous
154 tubercles on the inner side of the tight, ratio of pectoral vs. femoral seams (Bertolero et al.,
155 2011; Soler et al., 2012). Hybrids WHT x EHT displayed various combinations of phenotypic
156 characters and could not be identified with certainty (especially F2, unpublished genetic
157 results). Easily identifiable hybrids were brought to the Soptom rescue center.

158 Each tortoise was measured by strait carapace length (SCL), sexed when possible (small
159 immatures cannot be easily sexed), and weighted to the nearest gram. Individuals larger
160 than 100 mm in SCL were considered adult. Following blood, oral and nasal epithelium
161 sampling, individuals were subjected to clinical inspection (see below), and then they were
162 released at the place of capture, generally within 30 min. To ensure that researchers did not
163 spread pathogens and did not contaminate samples, they cleaned their hands and clothes
164 using Vircon spray 1% (Bayer©); equipment was cleaned with alcohol. Samples were stored
165 using one box per site.

166 Most individuals examined were free-ranging WHT (N=421; table 1). We identified 11
167 EHT: 2 free-ranging individuals introduced into WHT populations, 8 captive (pets), and one
168 vagrant. Thirty nine WHT x EHT easily identifiable hybrids were observed: 24 free-ranging, 9
169 captive and 6 vagrants. Twenty four spur-thighed tortoises were examined: 10 of them were
170 free-ranging and thus have been introduced into WTH populations, 12 were found in private

properties (pets), and 2 were vagrant. Finally, 1 captive marginated tortoise (*Testudo marginata*) was sampled.

Tissue sampling

Blood (0.4 - 0.7 ml) was collected from the subcarapacial plexus (Hernandez-Divers *et al.* 2002) using 1 ml syringes (Injekt-F – B Braun) and sterile needles (26G to 27G, Terumo Neolus, adjusted to the size of the animal). Subcarapacial plexus delivers various mixtures of blood and lymph, especially using needles larger than 27G (Bonnet *et al.* 2016); we did not notice such mixture during sampling. Blood was immediately placed in Sodium or Lithium heparin. Samples were gently homogenized and stored (max 4 hours) in ice-cooled containers until centrifugation (1500 rpm for 5 min). Plasma was stored at -25°C until analyses. Aliquots were distributed in two tubes: 50 µl for microbiological analysis and the rest for biochemical analyses.

We sampled oral and nasal epitheliums and mucus. We injected ~0.5 ml of sterile saline (0.9% sodium chloride, Lavoisier) to flush the nasal cavity. The resulting fluid was collected with a syringe (0.1 ml) in each nostril and immediately stored in a 0.5 ml sterile conical tube. Oral samples were collected with a brush (Cervibrush + LBC, Endocervical sampler, CellPath) inserted inside the oral cavity: choana and mucosal surfaces of the tongue and of the beak were targeted. Brushes were stored individually in a 0.5 ml sterile conical tube containing 0.3 ml of sodium chloride (0.9%) to avoid desiccation of the mucus. All samples were placed in ice-cooled containers in the field and stored at -25°C until analyses.

All samples were shipped frozen to Staaliches VetUAmt laboratory in Detmold, Germany, for analyses.

Pathogen screening

The presence of Testudinid herpesvirus (TeHV-serotypes 1 and 3) was assessed using both polymerase chain reaction (PCR) and induced antibody responses by serum neutralization test (SN) (Soares et al*.,* 2004; Salinas et al., 2011; Origgi, 2012). PCR test can be applied to TeHV-1 and TeHV-3 in any species of tortoise (Salinas et al. 2011). A PCR test was considered positive for TeHV when DNA of pathogens was detected in oral mucus. However, the mucus of individuals that do not present clinical signs is generally less rich in viral DNA compared to the mucus of individuals displaying clinical signs (Origgi, 2012). In addition, there is the possibility of false negatives related to the sampling methodology or the oscillating elimination of some viruses such as Herpesviruses (Marschang 2019). Thus, we used SN testing as a complementary method to detect the presence of TeHV types 1 and 3 circulating antibodies on plasma aliquots (Origgi, 2012).

The presence of *Mycoplasma agassizii* was assessed using PCR, no serological test being available in Europe during the study (2012-2016). Thus, PCR were used to detect active infection by *Mycoplasma* and TeHV (Soares et al., 2004).

Many tortoises were tested for both pathogens. Table 2 provides the numbers of tests performed on the different categories and taxa of tortoises.

Clinical inspection

All tortoises were visually inspected for clinical symptoms of URTD disease that are usually associated with mycoplasma and herpesvirus infections (Jacobson et al., 2014): keratitis, conjunctivitis, ocular and palpebral oedema, and nasal discharges (mucopurulent oculonasal discharge), necrotic spots on the oral mucosa and on the tongue, rhinitis, lethargy and low body condition (Brown et al., 2002; Berry and Christopher, 2001; Sandmeier et al., 2009).

Results

PCR testing revealed that seven free-ranging WHT (six adult females and one adult male) were TeHV positive (2.8 %, n=7/253), and that 3 sub-populations (i.e. sites) were concerned (tables 2 and 3). SN tests for TeHV were all negative (table 2).

A total of 52 individuals (species and subspecies pooled) were positive for mycoplasma DNA (prevalence Pr=9.2%) (table 2). Most were free-ranging individuals (n=39, 75%) and most populations were infected (15 of 18) leading to a mean prevalence of 8.7.5% (range: 3.4% to 25%; Table 3). Among free-ranging tortoises, most positive cases concerned WHT (n=34, 87%; Pr=8.7%) followed by spur-thighed tortoises (two subspecies added) (n=5, 13%, Pr=50%). All species pooled, 9 captive individuals were positive (Pr=9.9%) and were sampled in five private properties (among 21). They belonged to almost all species and subspecies found in the study area: 5 WHT (Pr=7.7%), 2 spur-thighed tortoises (Pr=18.2%), one *Testudo marginata* (Pr= 100%), and one hybrid (Pr= 16.6%). Among vagrants, 4 WHT were positives (Pr= 36.3%). Focusing on WHT (wild, captive and vagrant pooled) positive for mycoplasma, 23 were females (56.1%; Pr=9.6%, 23/238), 18 were males (43.9%; Pr=7.9%, 18/227), and two individuals were not sexed. No individual was positive for both pathogens.

Considering the whole sample, 28 individuals showed clinical symptoms of URTD; 4 displayed palpebral oedema, 1 with sunken eyes, 8 strong nasal discharges, and 14 abnormal mucus color (black, white or yellow), 1 exhibited abnormal mucus color + nasal discharge. Among these 28 animals, 3 were tested positive for mycoplasma (2 with nasal discharges, one with palpebral oedema) and none for TeHV. The general condition of the tortoises was apparently normal otherwise and none exhibited ocular discharge.

Discussion

This study reveals the first cases of herpesvirus contamination and considerably extends knowledge of mycoplasma infection occurrence (only one case previously known; Mathes et

al., 2001) in tortoises tested in natural populations in Europe (3 sub-populations for TeHV, 15 sub-populations for mycoplasma). These issues come sharply into focus when result obtained in captive and vagrant tortoises, native and exotic, are considered.

In this study, the high number of exotic tortoises observed vagrant (e.g., isolated individuals walking nearby private properties) or settled in WHT natural populations illustrate that undesired introduction of exotic pet tortoises is an ongoing process. Chelonian pet trades are intensifying worldwide (Stanford et al., 2020), including European tortoises with massive exports of EHT and spur-thighed tortoises from Turkey, Balkan countries or North Africa to France, Spain and Italy (Ljubisavljević et al., 2011). Our results reveal substantial infection rates by mycoplasma both in captive and vagrant individuals of different tortoise species sampled in the distribution range of the native species (WHT). This situation poses the problem of possible contamination from captive toward free-ranging tortoises, and perhaps conversely when free ranging WHT are illegally collected and brought in captivity.

The prevalence of mycoplasma infection was relatively similar in free-ranging and captive tortoises (Pr=8.7% and Pr=9.9% respectively); TeHV was not detected in captivity. Among pet tortoises, however, infection rate was higher in exotic and hybrid species (22.2%, 4/18) compared to captive WTH (7.7%, 5/65). In addition, high levels of infection were observed in exotic spur-thighed tortoises, both in captivity (18.2%, 2/11) and among individuals introduced in the field (44%, 4/9) compared to those observed in free-ranging WHT populations (13.5%, 43/318). This disequilibrium between exotic and native tortoise's infection levels suggests that a common exotic pet species may represent an important reservoir of mycoplasma.Herpesvirus is a virus with a low environmental persistence capacity, so it needs direct physical contact for its transmission between tortoises (Marschang 2019). The contribution of environmental contamination by Mycoplasma to

infection of tortoises could be overlooked mainly because the wall-less mycoplasmas were thought to be highly fragile. However, the ability of some mycoplasma species to form a biofilm in vitro has necessitated a reconsideration of their ability to survive in the environment (McAuliffe et al., 2006). As a consequence, environmental resistance conferred by biofilm formation might play a significant role in the continuous circulation of Mycoplasma among different individuals, which allows it to come into contact with more tortoises even in conditions of low population densities. Although preliminary, these results reinforce the notion that pet tortoises, and thus international trade of chelonians, may represent a health threat to native tortoises.

Finding that free-ranging tortoises can be infected by TeHV and mycoplasma in Europe, although worrying, is not fully surprising (negative SN tests for TeHV despite PCR positive tests suggest possible reactivation or a recent contact with the pathogen that has not yet generated a population immunity). Numerous assessments performed in captive tortoises maintained in cages or in outdoor enclosures (inaccurately categorized as “wild” or “free-living” individuals by various authors, leading to misleading conclusions) showed that many individuals belonging to different lineages were infected by various pathogens involved in URTD. For example, herpesvirus was detected by PCR in 16.3% of individuals from different tortoise species maintained in captivity in Belgium; a country voided of native chelonian however (Martel et al., 2009). Kolesnik et al. (2017) tested by PCR 1,015 captive tortoises and terrapins originating from different continents and kept captive (e.g. in cage, enclosure, park, garden) in different European countries; more than 42.1% were infected by mycoplasma and 8.0% were infected by herpesviruses. In Turkey, among 272 spur-thighed tortoises caught in the wild and kept in captivity (sometimes during years) to supply a breeding program designed to reinforce native populations, seroprevalences (SN test) were as follow: 37% positive for herpesvirus serotype-I, 5.5% for serotype-II, and 5.2% 5.2 % for

picorna-like 'X' virus and 4.9% for reovirus (X and reovirus) (Marschang and Schneider, 2007). Broadly similar PCR result of 36.7% was obtained with mycoplasma (Lecis et al., 2011). More generally, high infection prevalence for a wide range of pathogens has been observed in captive chelonians, notably in individuals intercepted during illegal trade (Brianti et al., 2010).

Herpesvirus infections have been documented more than 40 years ago in American and in European chelonians (Origgi, 2012). However, previous assessments performed two decades ago in tortoises actually sampled in the field in France and Morocco (*Testudo hermanni*, *T. graeca*) failed to detect herpesvirus (Mathes et al., 2001). A situation that contrasts with well-documented infections in free-ranging chelonians in North America (Jacobson, 1994; Berish et al., 2000; Jacobson et al., 2012; Kane et al., 2017; Weitzman et al., 2017; Lindemann et al., 2019; Orton et al., 2020). Further studies are needed to determine to what extent the presence of URTD pathogens results from recent transmissions from pet to wild tortoises in Europe, *versus* insufficient investigations in natural settings. Unfortunately, monitoring infection prevalence and severity is difficult in the field. Underestimations are likely because infected animals that may die are quickly removed prior to sampling. Further, weakened sick tortoises may well remain concealed and may not be easily detected in the field.

Infection risks and diseases

Lack of severe clinical signs should be treated with caution. Silent TeHV and Mycoplasma infections have been reported in tortoises (Orrigi, 2012; Withfield et al., 2018). Multiple strains of pathogens circulate worldwide (Salinas et al., 2011; Jacobson et al., 2014; Kolesnik et al., 2017), they continuously evolve while pathology and coinfections risks increase with pathogen diversity (Kari et al., 2008). Thus, constant introductions of infected pets into

natural populations represent a threat through the emergence of infectious diseases, especially if exotic tortoises tolerate and thus carry pathogens that can cause outbreaks in less-tolerant native species (Berish et al., 2010; Jacobson et al., 2014; Whitfield et al., 2018; DiGeronimo, 2019; Goessling et al., 2019). Environmental factors, like seasonal variations of immunity and physiological stress can perturb equilibriums with pathogens, tipping the balance toward diseases; especially in case of multiple infections (Sandmeier et al., 2013; Goessling et al., 2016).

In European tortoises, the presence of herpesvirus and the high prevalence of mycoplasma in natural populations were only suspected prior to the current study. But the paucity of information regarding possible health impact of TeHV, in combination with mycoplasma, along with the spectre of emerging serious diseases caused by picornavirus in captive Hermann's tortoises in Spain for example (Martinez-Silvestre et al., 2020), prompts for further studies.

Perspectives and recommendations

French continental populations of Hermann's tortoise represent relicts of the distribution of a previously widespread and abundant species. It is crucial to not add infectious burden to the multitude of existing threats (e.g. habitat fragmentation, sprawling urbanization, frequent fires, illegal harvesting, wild-boars, dogs). Both the prevalence and demographic impact of URTD in the field should be carefully monitored. Pet owners should be informed that reproducing tortoises in captivity and releasing individuals in the field may threaten wild populations. Exotic tortoises should be removed from native populations. Long-term mark-recapture surveys should be coupled with health checking, including monitoring of most deleterious strains of TeHV (Gandar et al., 2015). Sanitary protocols are needed to handle free-ranging tortoises; nasal and oral secretions, feces, sperm and even urine can be

contaminating (Origgi et al., 2012). Similarly, strict protocols are needed during conservation translocations; notably because individuals often travel long distances before settling (Pille et al., 2018), while stress associated with release may promote virus reactivation from latent infection (Griffith, 1993; Martel et al., 2009; Jacobson et al., 2012). Furthermore, captive individuals carrying a wide range of pathogens and parasites represent additional risks to URD transmission and should be treated specifically (Ahne 1993; Chávarri et al. 2012).

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Figure 1: Sampling sites inside the French continental distribution range of WHT.

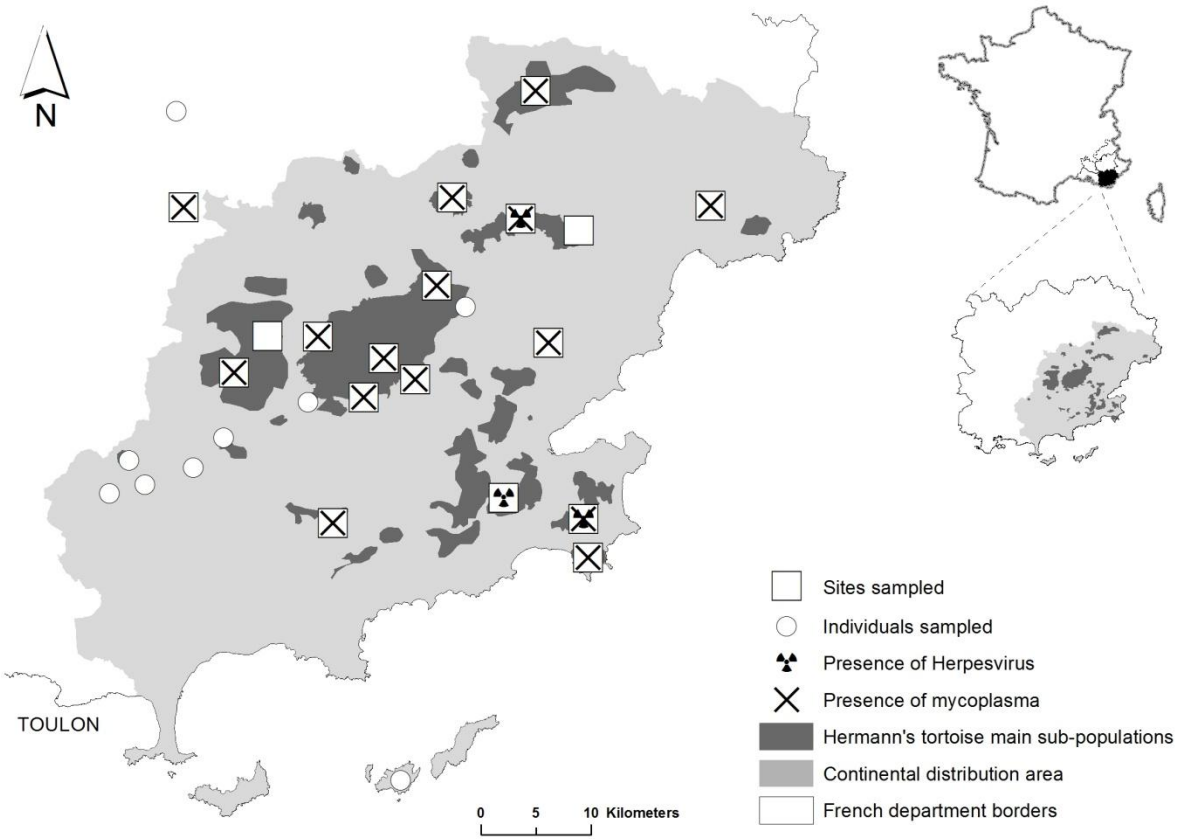
N
TOULON
0 5 10 Kilometers
Sites sampled
Individuals sampled
Presence of Herpesvirus
Presence of mycoplasma
Hermann's tortoise main sub-populations
Continental distribution area
French department borders

Table 1: Numbers of individual tortoises sampled in function of species and subspecies (taxon) and in function of the situation where the animals were found (free-ranging, captive or vagrant, see text for details). *Testudo hermanni hermanni* is named WHT in this study, *T.h. boettgeri* EHT. Different subspecies of the spur-thighed tortoises (*T. graeca*) are not distinguished in the main text. WHT x EHT stands for hybrids.

	Free-ranging	**Captive (pet)**	**Vagrant**	**TOTAL**
T. h. hermanni	421	65	11	497
T. h. boettgeri	2	8	1	11
T. g. ibera	1	-	-	1
T. g. sp	9	12	2	23
T. marginata	-	1	-	1
WHT x EHT	24	9	6	39
TOTAL	**457**	**95**	**20**	**572**

Table 2: results from the PCR tests for herpesvirus and mycoplasma infections according to taxon and in function of the situation where the tortoises were found. . TeHV stands for Herpesvirus, SN for Serum Neutralization, for PCR polymerase chain reaction test, Mycoplasma for *Mycoplasma agassizii*. Most individuals were tested for both pathogens with PCR (TeHV + Myc).n" indicate the number of sampled individuals, "+" indicate the number of positive individuals, percentages to the total number of sampled tortoises per taxon and situation are provided into bracket.

		Free-ranging		Captive (pet)		Vagrant		TOTAL	
Test	**Taxon**	n	+	n	+	n	+	n	+
TeHV SN	*T. h. hermanni*	253	0	48	0	8	0	309	0
	T. h. boettgeri	2	0	6	0	1	0	9	0
	T. g. ibera	1	0	0	0	0	0	1	0
	T. g. sp	8	0	10	0	2	0	20	0
	T. marginata	0	-	0	-	0	-	0	-
	WHT x EHT	32	0	6	0	3	0	41	0
	TOTAL	**296**	**0**	**70**	**0**	**14**	**0**	**380**	**0**
TeHV PCR	*T. h. hermanni*	253	7 (2.8%)	58	0	10	0	321	7
	T. h. boettgeri	2	0	7	0	1	0	10	0
	T. g. ibera	1	0	0	0	0	0	1	0
	T. g. sp	9	0	12	0	2	0	23	0
	T. marginata	0	0	1	0	0	0	1	0
	WHT x EHT	35	0	4	0	5	0	44	0
	TOTAL	**300**	**7 (2.3%)**	**82**	**0**	**18**	**0**	**400**	**7 (1.7%)**
Mycoplasma PCR	*T. h. hermanni*	391	34 (8.7%)	65	5 (7.7%)	11	4 (36.3%)	467	43 (9.2%)
	T. h. boettgeri	2	0	8	0	1	0	11	0
	T. g. ibera	1	1 (100%)	0	-	0	-	1	-
	T. g. sp	9	4 (44.4%)	11	2 (18.2%)	2	0	22	6 (27.3%)
	T. marginata	0	-	1	1 (100%)	0	-	1	-
	WHT x EHT	45	0	6	1 (16.7%)	7	0	58	1 (1.7%)
	TOTAL	**448**	**39 (8.7%)**	**91**	**9 (9.9%)**	**21**	**4 (19.0%)**	**560**	**52 (9.2%)**
TeHV + Myc PCR	*T. h. hermanni*	250	0	58	0	10	0	318	0
	T. h. boettgeri	2	0	7	0	1	0	10	0
	T. g. ibera	1	0	0	0	0	0	1	0
	T. g. sp	9	0	11	0	2	0	22	0
	T. marginata	0	0	1	0	0	0	1	0
	WHT x EHT	35	0	4	0	5	0	44	0
	TOTAL	**297**	**0**	**81**	**0**	**18**	**0**	**396**	**0**

Table 3 results from the PCR tests for herpesvirus and mycoplasma infections in the different sites sampled (N=457 free-ranging individuals); Each site hosts a free-ranging population of WHT and may contained individual from other Taxon. The site named

576 “divers“ indicated Individuals randomly sampled throughout the area. “n” indicate the

577 number of sampled individuals, “+” indicate the number of positive individuals),,

578 percentages to the total number of sampled tortoises per site are provided into bracket.

579

	TeHV - PCR		*Mycoplasma*-PCR	
Sites	**n**	**+**	**n**	**+**
3 caps	12	0	18	2 (11.1%)
La Pardiguière	28	0	28	1 (3.6%)
Callas	17	0	24	4 (16.7%)
Carcès	10	0	10	2 (20%)
Cogolin-La Môle	20	4 (20%)	23	0
Estérel	18	0	28	5 (17.9%)
Flassans calcaire	13	0	21	0
Lambert	15	0	23	1 (4.3%)
Le Muy - St Luen	12	1 (8.3%)	21	2 (9.5%)
Les Arcs	13	0	12	3 (25%)
Neuf riaux / Est PDM	11	0	29	1 (3.4%)
Ramatuelle	9	2(22.2%)	16	1 (6.3%)
Redon	18	0	24	2 (8.3%)
Reserve National des Maures	18	0	21	3 (14.3%)
Roquebrune sur Argens	11	0	17	0
Les Mayons	25	0	60	5 (8.3%)
Sainte-Maxime	15	0	18	3 (16.7%)
Vidauban	19	0	35	2 (5.7%)
"Divers"	16	0	20	2 (10%)
TOTAL	300	7 (2.3 %)	448	39 (8.7%)

580

581

582

583