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Testudine Intranuclear Coccidiosis (TINC) in Europe: A Retrospective Study, 2022–24

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Abstract

Testudine intranuclear coccidiosis (TINC) infects a wide range of chelonian species and can cause systemic disease and death. Samples submitted for TINC detection by PCR to a commercial diagnostic laboratory in Germany between 2022 and 2024 were retrospectively evaluated. Of 325 samples tested, 47 (14.46%) were positive for TINC, including 40 Testudinidae (of 269 tested, 14.87%) and 7 of unknown species (of 34, 20.59%), whereas none of the samples submitted from other families ($n = 22$) tested positive. The highest detection rates were found in Burmese star tortoises (*Geochelone platynota*; 7/15, 46.7% positive), Hermann's tortoises (*Testudo hermanni*; 3/13, 23.1%), and radiated tortoises (*Astrochelys radiata*; 8/36, 22.2%). Positive samples were submitted from Austria, Belgium, Czech Republic, Germany, Hungary, Italy, The Netherlands, Spain, and the UK. Coinfection with TINC and mycoplasma was detected in 15 animals ($n = 207$ tested for both), coinfection with a herpesvirus and TINC was detected in 3 animals ($n = 188$), and coinfection with a torchivirus and TINC was detected in 2 animals ($n = 123$). The TINC detection rate was significantly higher than that previously reported in Europe for 2014–15 (47/325, 14.5% compared with 27/1,010, 2.7%; $P < 0.001$). TINC is detected regularly in diagnostic samples from tortoises in managed care in Europe and should be considered as a differential diagnosis for nonspecific disease and for quarantine examinations in these animals.

Keywords: *Astrochelys radiata*, Europe, intranuclear coccidia, radiated tortoise, *Testudo* spp., testudine intranuclear coccidiosis

Introduction

There are >70 coccidia that have been described in chelonians so far, and it is likely that many more are yet to be discovered (Duszynski and Morrow, 2014). The first intranuclear coccidian in chelonians was detected in 1994 in radiated tortoises (*Astrochelys radiata*) in the United States (Jacobson *et al.*, 1994). The associated disease is generally referred to as testudine intranuclear coccidiosis (TINC). The causative pathogen has not yet been classified, and details regarding its life cycle remain unknown. However, sequence analyses of a portion of the genome indicate that these parasites cluster basal to previously described eimeriorine coccidia (Innis *et al.*, 2007; Hofmannová *et al.*, 2019).

A wide range of chelonian species are known to be susceptible to TINC, and TINC is considered an emerging infectious disease in tortoises (Gibbons and Steffes, 2013; Stilwell *et al.*, 2017; Adamovicz *et al.*, 2020; Laghzaoui *et al.*, 2021). The causative parasite has been found in a wide variety of species, including mostly tropical tortoises (e.g., leopard tortoises [*Stigmochelys pardalis*], Indian star tortoises [*Geochelone elegans*], spider tortoises [*Pyxis arachnoides*], and red-footed tortoises [*Chelonoidis carbonarius*]), but also in Emydidae (e.g., box turtles [*Terrapene carolina carolina*] and Geoemydidae (e.g., Arakan forest turtles [*Heoseyms depressa*]) (Garner *et al.*, 2006; Alvarez *et al.*, 2013; Kolesnik *et al.*, 2017a; Stilwell *et al.*, 2017). Detection has been reported in chelonians in North America, Europe, Asia, and Africa (Alvarez *et al.*, 2013; Kolesnik *et al.*, 2017a; Laghzaoui *et al.*, 2021).

Disease associated with TINC is often nonspecific and can range from anorexia, lethargy, and weight loss to respiratory signs, oral lesions, cloacal swelling, ascites, and systemic disease (Garner *et al.*, 2006; Innis *et al.*, 2007; Schmidt *et al.*, 2008; Hofmannová *et al.*, 2019). Experimental studies have supported the wide host range of the pathogen and also indicated possible differences in susceptibility to disease caused by these intranuclear coccidia between different tortoise species (Hofmannová *et al.*, 2019).

A study published in 2017 described screening of diagnostic samples from 1,010 chelonians in human care in Europe for TINC (Kolesnik *et al.*, 2017a). The samples in that study were submitted for testing for a variety of other pathogens by PCR to a veterinary diagnostic laboratory between 2014 and 2015. In total, 2.7% of those samples were PCR positive for TINC. That study expanded the known host range for this

pathogen to include several European tortoise species (*Testudo* spp.). The species in which TINC were most commonly detected included radiated tortoises (5/22, 22.7% positive), Indian star tortoises (2/17, 11.8% positive), African spurred tortoises (*Centrochelys sulcata*; 2/24, 8.3% positive), leopard tortoises (4/51, 7.8% positive), and marginated tortoises (*Testudo marginata*; 3/40, 5.0% positive).

Diagnosis of TINC is most often accomplished by PCR targeting a portion of the 18S rRNA gene (Alvarez *et al*., 2013). Blood samples; cloacal, oral and nasal swabs; and lavages, feces, and tissues have been used for diagnostic testing (Innis *et al*., 2007; Stilwell *et al*., 2017; Kolesnik *et al*., 2017a). Cloacal shedding of oocytes has been described, but oocytes can be difficult to detect in feces due to their fragility (Hofmannová *et al*., 2019). Because shedding may depend on host species and stage of infection, combined conjunctival, oral, and cloacal swabs, together with nasal discharge if present, have been recommended for antemortem diagnostic testing (Wellehan *et al*., 2022).

The aim of this study was to retrospectively evaluate the results of PCR testing for TINC in samples submitted to a veterinary diagnostic laboratory between 2022 and 2024 and to compare the results with those from the same laboratory published previously (Kolesnik *et al*., 2017a).

Materials and Methods

Diagnostic samples that had been submitted to a veterinary diagnostic laboratory (Laboklin GmbH & Co. KG, Bad Kissingen, Germany) between January 2022 and December 2024 were evaluated. All samples had been submitted by veterinarians or owners, and the submitters had specifically requested testing for TINC. Samples were processed as described previously (Kolesnik *et al*., 2017a). In brief, DNA and RNA were extracted within 24 h of arrival in the laboratory by using a commercial kit (MagNA Pure 96 DNA and Viral NA Small Volume Kit, Roche, Mannheim, Germany). The coccidia associated with TINC were detected by real-time PCR targeting a portion of the 18S rRNA gene (Alvarez *et al*., 2013). If requested, additional PCR testing for the detection of mycoplasmas (Brown *et al*., 1999; Faulhaber *et al*., 2025), herpesviruses (VanDevanter *et al*., 1996; Teifke *et al*., 2000; Murakami *et al*., 2001), and torchiviruses (Kolesnik *et al*., 2017b) was also done on the same samples.

Statistical analysis was carried out with the SAS® statistical software, version SAS OnDemand for academics (SAS Institute Inc., Cary, NC, USA) by using Fisher's exact tests. A $P < 0.05$ was considered significant.

Results

In total, 325 samples were submitted between 2022 and 2024 for testing for TINC (Table 1).The species from which the sample was collected was not always provided, but samples from at least 39 species were tested. The majority of samples (269/325, 82.77%) were from tortoises in the family Testudinidae, including at least 28 species. A small number of samples were also submitted from chelonians in the families Emydidae, Geoemydidae, Podocnemididae, and Trionychidae. In 34 cases, no information was available on the family or species from which the sample was obtained.

Intranuclear coccidia were detected in 47 (14.46%) of the 325 samples tested, including 40 Testudinidae (of 269 tested, 14.87%) and 7 of unknown species (of 34, 20.59%). None of the samples from Emydidae, Geoemydidae, Podocnemididae, or Trionychidae (of 22 tested) were positive for TINC (Table 1). A comparison of TINC detection in Testudinidae with detection in all other families combined showed no statistically significant difference ($P = 0.053$). Of those samples for which the species was known, TINC was detected in 11 different species in total.

Ten or more samples were tested from eight different tortoise species in seven genera (Table 1). TINC was detected in samples from six (75%) of these eight species and six (85.7%) of the seven genera. The differences in detection rates between these species and genera were statistically significant (both $P < 0.001$).

The samples tested had been submitted from 16 European countries. Positive samples were submitted from Austria (1/26, 3.85% positive), Belgium (1/2, 50%), Czech Republic (3/12, 25%), Germany (2/84, 2.34%), Hungary (3/5, 60%), Italy (9/21, 42.86%), The Netherlands (2/4, 50%), Spain (7/33, 21.21%), and the UK (1/41, 2.44%). The differences in detection rates between countries was significant ($P < 0.001$).

Mixed infections were found in several cases. In total, 207 samples were also tested for mycoplasma, 83 (40.1%) of which were positive. Of these, TINC was also detected in 15 (18.07%). There was a significant relationship between infection with mycoplasma and TINC ($P = 0.048$). Samples from 188 animals were also tested for herpesviruses: 3 (1.6%) were positive, and TINC was detected in 1 (33.3%) of these. The relationship between herpesvirus and TINC detection was not significant ($P = 0.334$). Samples from 123 animals were also tested for torchiviruses: 4 (3.35%) were positive, and TINC was detected in 2 (50%) of these. The relationship between torchivirus and TINC detection was not significant ($P = 0.092$).

A comparison of the results from 2022 to 2024 with those of a previous study in which samples submitted for routine PCR testing for other infectious agents were additionally tested for TINC in 2014 and 2015 (Kolesnik *et al*., 2017a) showed several significant differences (Table 2). The overall detection rate was significantly higher in 2022–24 (14.46%) than in 2014–15 (2.7%) ($P < 0.001$). Looking at detection rates in Testudinidae only, the difference was also significant: 2.8% in 2014–25 and 14.87% in 2022–24 ($P < 0.001$).

Discussion

Testudine intranuclear coccidiosis is detected regularly in tortoises in Europe (Schmidt *et al*., 2008; Kolesnik *et al*., 2017a; Hofmannová *et al*., 2019). The actual prevalence of TINC among chelonians in managed care is not known. A previous study examining TINC detection rates in chelonians in managed care focusing mostly on Europe found TINC in 2.7% (27/1,011, 1,010 of which were from Europe)

Table 1. Detection of testudine intranuclear coccidiosis (TINC) in samples submitted to a commercial veterinary diagnostic laboratory in Europe between January 2022 and December 2024: species tested and TINC detection rates.

Family	Species	No. tested	No. positive (% positive)
Testudinidae	All	269	40 (14.9)
	Aldabra giant tortoise (*Aldabrachelys gigantea*)	13	0
	Radiated tortoise (*Astrochelys radiata*)	36	8 (22.2)
	Ploughshare tortoise (*Astrochelys yniphora*)	2	0
	African spurred tortoise (*Centrochelys sulcata*)	32	2 (6.3)
	Red-footed tortoise (*Chelonoides carbonarius*)	27	4 (14.8)
	Yellow-footed tortoise (*Chelonoidis denticulatus*)	7	1 (14.3)
	Galapagos giant tortoise (*Chelonoidis niger*)	3	0
	Chelonoidis sp.	1	0
	Angulate tortoise (*Chersina angulata*)	1	0
	Indian star tortoise (*Geochelone elegans*)	7	0
	Burmese star tortoise (*Geochelone platynota*)	15	7 (46.7)
	Parrot-beaked dwarf tortoise (*Homopus areolatus*)	3	0
	Elongated tortoise (*Indotestudo elongata*)	5	0
	Serrated hingeback tortoise (*Kinixys erosa*)	6	1 (16.7)
	Home's hingeback tortoise (*Kinixys homeana*)	1	0
	Speke's hingeback tortoise (*Kinyxis spekii*)	1	0
	Pancake tortoise (*Malacochersus tornieri*)	1	0
	Brown tortoise (*Manouria emys*)	6	0
	Impressed tortoise (*Manouria impressa*)	5	2 (40)
	Madagascar spider tortoise (*Pyxis arachnoides*)	3	0
	Madagascar flat-tailed tortoise (*Pyxis planicauda*)	6	1 (16.7)
	Pyxis sp.	2	0
	Leopard tortoise (*Stigmochelys pardalis*)	44	8 (18.2)
	Mediterranean spur-thighed tortoise (*Testudo graeca*)	11	0
	Hermann's tortoise (*Testudo hermanni*)	13	3 (23.1)
	Horsfield's tortoise (*Testudo horsfieldii*)	2	0
	Egyptian tortoise (*Testudo kleinmanni*)	4	1 (25)
	Marginated tortoise (*Testudo marginata*)	3	0
	Testudo sp.	2	0
	Tortoise, species unknown	7	2 (28.6)
Emydidae	All	7	0
	European pond turtle (*Emys orbicularis*)	4	0
	Yellow-blotched map turtle (*Graptemys flavimaculata*)	1	0
	Box turtle (*Terrapene* sp.)	1	0
	Pond slider (*Trachemys scripta*)	1	0
Geoemydidae	All	12	0
	Indochinese box turtle (*Cuora galbinifrons*)	1	0
	Southern Vietnamese box turtle (*Cuora picturata*)	4	0
	Annam leaf turtle (*Mauremys annamensis*)	3	0
	Mediterranean turtle (*Mauremys leprosa*)	2	0
	Chinese striped-necked turtle (*Mauremys sinensis*)	1	0
	Tricarnate hill turtle (*Melanochelys tricarinata*)	1	0
Podocnemididae	Madagascan big-headed side-necked turtle (*Erymnochelys madagascariensis*)	2	0
Trionychidae	Chinese soft-shelled turtle (*Pelodiscus sinensis*)	1	0
Unknown		34	7 (20.6)
Total		325	47 (14.5)

of the chelonians tested (Kolesnik *et al*., 2017a). This is in contrast to the 14.46% (47/325) positive animals found in our study. There are several possible reasons for the increase in detection rate between the study in 2014 and 2015 (Kolesnik *et al*., 2017a) and our study. It is possible that infection with TINC is spreading among tortoises in Europe and the actual prevalence in managed collections is increasing. It has been suggested that TINC is an emerging infectious disease of tortoises (Gibbons and Steffes, 2013; Adamovicz *et al*., 2020), and the increasing detection rate found could reflect

Table 2. Comparison of results between a previous study screening chelonians for the presence of testudine intranuclear coccidiosis (TINC) by PCR in 2014–15 (Kolesnik *et al.*, 2017a) and 2022–24 (this study) for species with ≥10 tested in at least one of the two studies.

Species	2014–15		2022–24		*P* value
	n	% positive	*n*	% positive	
Aldabra giant tortoise (*Aldabrachelys gigantea*)	8	0	13	0	ND
Radiated tortoise (*Astrochelys radiata*)	22	22.7	36	22.2	0.964
African spurred tortoise (*Centrochelys sulcata*)	24	8.3	32	6.25	0.765
Red-footed tortoise (*Chelonoides carbonarius*)	3	0	27	14.81	ND
Galapagos giant tortoise (*Chelonoidis niger*)	10	0	3	0	ND
Indian star tortoise (*Geochelone elegans*)	17	11.8	7	0	ND
Burmese star tortoise (*Geochelone platynota*)	1	0	15	46.67	ND
Brown tortoise (*Manouria emys*)	13	0	6	0	ND
Leopard tortoise (*Stigmochelys pardalis*)	51	7.8	44	18.18	0.130
Mediterranean spur-thighed tortoise (*Testudo graeca*)	132	0	11	0	ND
Hermann's tortoise (*Testudo hermanni*)	194	2	13	23.08	<0.001
Horsfield's tortoise (*Testudo horsfieldii*)	83	0	2	0	ND
Marginated tortoise (*Testudo marginata*)	40	5	3	0	ND

ND, not determined.

that status. However, the criteria for testing differed between the two studies. In Kolesnik *et al.* (2017a), samples had been submitted for a variety of reasons for testing for a wide range of infectious agents, and testing for TINC was often done without the specific request of the submitting veterinarian. In our study, only samples that were specifically submitted for testing for TINC were included. In addition, TINC may be better known among veterinarians in Europe now than a decade ago, leading to more focused diagnostic testing. The higher detection rate is therefore likely to reflect specific criteria used for sample selection as well as bias caused by sampling in cases of known infection.

Evaluation of detection rates in specific species showed that TINC infection rates have remained high in radiated tortoises in Europe in recent years. The detection rate in this species was 22.7% (5/22) in 2014–15 (Kolesnik *et al.*, 2017a) and 22.2% (8/36) in our study. Intranuclear coccidiosis was first described in radiated tortoises in the United States (Jacobson *et al.*, 1994), and reports in this species have since been published repeatedly (Garner *et al.*, 2006; Schmidt *et al.*, 2008; Kolesnik *et al.*, 2017a). The first report of TINC in European tortoises (*Testudo* sp.) was published in 2017 (Kolesnik *et al.*, 2017a), in the previous study screening samples from chelonians in Europe. In that study, TINC was detected at low rates in Hermann's (*T. hermanni*; 2/194, 1%) and marginated (2/40, 5%) tortoises, but not in any of the spur-thighed (*T. graeca*; $n = 132$) or Horsfield's (*T. horsfieldii*; $n = 83$) tortoises tested. In our study, TINC was detected in 23.1% (3/13) of the Hermann's tortoises tested and in 1 of the 4 (25%) Egyptian tortoises (*T. kleinmanni*) tested, but in none of the spur-thighed ($n = 11$), marginated ($n = 3$), or Horsfield's ($n = 2$) tortoises tested. An experimental infection of juvenile spur-thighed, Hermann's, and Horsfield's tortoises led to no clinical disease in the inoculated Hermann's and Horsfield's tortoises, whereas the spur-thighed tortoises developed anorexia, weight loss, and lethargy (Hofmannová *et al.*, 2019). All three species shed oocytes over an extended period postinoculation.

Whereas significant differences were found in detection rates between samples submitted from various countries, this is likely influenced by sampling bias, because veterinarians in different countries may choose to test samples based on different criteria. The previous study evaluating samples tested in 2014 and 2015 found TINC in samples from the following European countries: Austria, Denmark, France, Italy, Germany, Portugal, Spain, Sweden, Switzerland, and the UK (Kolesnik *et al.*, 2017a). In our study, positive samples were submitted from Austria, Belgium, Czech Republic, Germany, Hungary, Italy, Netherlands, Spain, and the UK. Together, this indicates a broad dissemination of TINC among tortoises in human care across the European continent.

Mixed infections with other pathogens were found in numerous cases in this study. Although a significant relationship was found between mycoplasma and TINC infection, the role of coinfection on susceptibility to disease is not known. The previous study of diagnostic samples from chelonians in Europe found no significant relationship between TINC infection and other infectious agents (Kolesnik *et al.*, 2017a). Mycoplasma infections are found regularly in tortoises in Europe (Kolesnik *et al.*, 2017b). It is possible that the statistical relationship calculated between these pathogens is a result of sampling bias rather than an actual influence of the various infectious agents among one another. Testing for both mycoplasma and TINC are likely influenced by the clinical signs observed in the animals (which were not known in this study) as well as other factors guiding diagnostic decision making of the treating veterinarians. Studies on the role of coinfections on both susceptibility to TINC infection and development of disease would be of interest.

There were several limitations to this study. The use of samples submitted to a commercial laboratory for diagnostic

testing meant that sample collection, sample type, and reasons for sampling were not defined and could have differed between samples. Sample quality and transport to the lab could have influenced the results, although all samples were processed within 24 h once they reached the lab. Information on clinical status or history of sampled animals was not available, and therefore clinical relevance of infection could not be evaluated. In some cases, the host species was not provided.

This study indicates that TINC is present in a significant number of tortoises in human care in Europe. There is some evidence that prevalence among tortoises in managed care may be increasing, although additional study is needed to verify this finding. Because TINC can significantly impact the health of affected chelonians, testing for this pathogen should be considered when animals are moved or during quarantine. In addition, TINC should be considered as a differential diagnosis in tortoises with nonspecific signs of disease in Europe.

Conflict of interest: The authors are both employed by a commercial veterinary diagnostic laboratory. This employment did not influence study design, interpretation, or publication.

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