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A clinicopathological analysis of Ivermectin-induced toxicity in eight Hermann's Tortoises treated for tick infestation

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ABSTRACT

This study presents a clinico-pathological examination of Ivermectin-induced toxicity in eight Hermann's tortoises, administered to address tick infestation. Subsequently, nervous signs were observed, leading to the demise of five tortoises. Clinical manifestations included inappetence, weakness, diarrhea, tremors, and paralysis preceding death. Postmortem examination revealed hemorrhagic lung tissues, fatty liver, and significant tick infestation. Laboratory analysis detected *Borrelia spp*. in blood smears, *Oxyuris spp*. eggs and cysts in fecal samples, and *Escherichia coli* and *Salmonella enterica* in microbial cultures. Histopathological analysis indicated hepatic vacuolar degeneration. The correlation between clinical, pathological, and histopathological findings suggests acute infection and drug toxicity, particularly Ivermectin toxicity. The tortoises, initially weighing an average of 900 g, were pets at Niger Resort Minna and were presented to the Nigerian Police Veterinary Hospital, Garki 2 on the 27th of May 2023, after exhibiting symptoms of inappetence and severe diarrhea following the administration of 1% Ivermectin (Ivanor from Jubaili Animal Health Ltd) at a dose rate of 0.04 mg/kg. Three tortoises died shortly after administration, with two succumbing within hours.

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Introduction

Ticks, nematodes, and insects are examples of parasitic organisms that pose a serious threat to human and animal wellbeing [1–3]. When it comes to the parasites obtaining the nutrition and physiological requirements they need from their hosts, there are numerous significant host-parasite specificity [3]. In real sense, parasites not only spread illnesses and infections but also severely reduce production and restrict the growth of their host, which results in enormous losses from an economic point of view [4–6] and occasionally results in death.

As terrestrial reptiles inhabiting grassy landscapes, Hermann's Tortoises are susceptible to tick infestations, similar to other fauna in their habitat. Ticks are the primary ectoparasites that commonly affect tortoises, showing preferences for different hosts depending on species and life stages [7]. Because of the tortoise's habitat, they are particularly susceptible to infestations by ticks of the *Hyalomma* genus [7,8]. *Hyalomma* ticks are identified as the predominant tick species affecting tortoises in regions including the Mediterranean, Middle East, Black Sea, and Central Asia [7]. These ticks have been found to carry various zoonotic pathogens, including the Crimean-Congo hemorrhagic fever virus. In addition, *Hyalomma* ticks can harbor other pathogens such as *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Coxiella burnetii*, which also have zoonotic potential [7].

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Avermectins are a class of 16 distinct chemical compounds that fall under the macrocyclic lactones medication category of which Ivermectin is part of them. They have been shown to have well-documented and proven effects on insects, worms, and ticks [9–11]. They were first created through the fermentation process by Gram-positive soil-dwelling bacteria as well as by members of the common group known as streptomyces, actinomycetes, and avermitilis [12–15]. Avermectins bind to ligand-gated chloride channels in both vertebrates and invertebrates, which has a toxic impact [16]. While Ivermectin is a naturally occurring substance, it is also employed in medicine [13]. It is used to treat a variety of parasites in livestock and pets in addition to its primary usage against mites, nematodes, and other economically significant agricultural pests [17,18]. Its mode of action involves binding with the chloride channels in the target organism's central nervous system, which is activated by glutamate and/or γ-aminobutyric acid (GABA). This inhibits neuromuscular and inter-neural transmission, causing spastic, uncoordinated gait, paralysis, and eventually death [10].

The management and growth of livestock are significantly impacted by the use of anthelmintics, such as Ivermectin, in modern chemotherapeutic treatments for parasitic infections [19]. However, overuse of these antiparasitic medications, particularly Ivermectin, can result in a number of issues, such as increased risk to human health from food poisoning and contamination, an increase in populations of helminth resistant to Ivermectin, and the development of intoxication that could result in drug overdose death [20]. Although, the majority of anthelmintics have broad safety margins, and using these medications may occasionally result in intoxication episodes [19]. Some of the main causes leading to overdose cases include administering the same number of drugs to all animals using the initially established or estimated average weight of these animals, using defective dosing equipment, failing to follow or adequately interpret label instructions, and the possibility of drug resistance or overestimation of animal body weight to drug ratio [21]. Lethality data, such as 10-d LC50 values, are often provided by the few studies that describe Ivermectin's toxicity to invertebrate species [22–24]. Strong antiparasitic medications called avermectins can be extremely poisonous to animals. As demonstrated in a number of instances, this can even cause an animals' death by harming its numerous organs [25–27] as stated earlier on.

Numerous papers and works have been reported on the occurrence of nephrotoxicity and other pathological alterations caused by avermectins. For instance, in pigs and dogs, Ivermectin induces nephrotoxicity by raising creatinine levels and lowering glucose and protein synthesis [28,29]. In addition to increased levels of nitrogenous substances in the body caused by this group of drugs, such as serum creatinine, urea, and uric acid in the blood, this oxidative damage causes histopathological changes such as interstitial nephritis, glomerular damage, interstitial infiltration areas of round cells, and tubular necrosis [26,30,31]. Also, the majority of foods, medications, and poisons that are present in the body are catabolized and neutralized by the liver, which is the primary organ responsible for detoxification [32]. Toxins and medications from this drug can damage the liver and cause total liver failure, which can result in the animal's death from severe, irreversible internal organ damage. Regardless of the route of drug administration, avermectins are easily bound to fat as well and are discovered in the liver of animals where they are mostly concentrated [33,34]. By influencing the liver enzymes, changing the hepatocytes' mitochondrial bioenergetics, leading to oxidative stress, and aiding autophagy in the liver tissues of the treated animals [35–37].

Hermann's Tortoises, although relatively rare in Nigeria, have become popular pets due to their gentle nature and relatively low-maintenance care requirements. However, like all animals, they are susceptible to health issues, with parasitic infestations being a significant concern. These infestations, caused by various ectoparasites and endoparasites, pose a considerable threat to the well-being of tortoises. Ticks, for example, are common ectoparasites found on tortoises, often congregating in large numbers. They tend to target areas of mildly keratinized and soft skin, such as the base of the neck and proximal extremities. In addition, ticks can attach to the seams between the scutes of the plastron, taking advantage of the thin layer of keratin for easy penetration and adherence [38]. Such infestations can severely impact the tortoises' quality of life and overall health.

In addressing these infestations, veterinarians frequently turn to Ivermectin, a broad-spectrum anti-parasitic medication effective against both internal and external parasites in reptiles and various other animals. While generally considered safe and effective, the use of Ivermectin in tortoises can sometimes lead to severe adverse effects, particularly in chelonians. Studies have shown that

Ivermectin can be toxic to certain species of chelonians, potentially crossing the blood–brain barrier or acting on peripheral neurotransmitters like GABA [39]. Toxic effects may include paresis, paralysis, and even mortality, especially due to respiratory muscle paralysis. Consequently, literature and experts caution against the use of Ivermectin in tortoises due to the risk of morbidity and death [40].

Although research on Ivermectin toxicity in chelonians has primarily focused on species such as leopard tortoises, red-footed tortoises, eastern box turtles, and red-eared sliders [41], with varying toxicity levels observed based on species, dosage, and dosing intervals, comprehensive studies on Hermann's Tortoises remain limited. Paresis and paralysis are the primary signs associated with Ivermectin toxicity in chelonians [41], but these effects can be mitigated by administering lower dosages and adhering to correct dosing intervals[40,41].

This study investigates the clinicopathological aspects of Ivermectin-induced toxicity in eight Hermann's Tortoises, shedding light on the post-mortem and clinicopathological changes observed with the administration of Ivermectin in these reptiles. By examining the outcomes of such toxicity, the study aims to emphasize the critical importance of accurate diagnosis and the implementation of appropriate management strategies especially drug administrations in treating a parasitic infection or infestation in Hermann's Tortoises to protect the health and well-being of these beloved pets.

Methods

Five of the eight Hermann's tortoises weighing 900 g on average before death, that were kept as pets at the Niger Resort Minna were presented to the postmortem unit of the Nigeria Police Veterinary Hospital, Garki 2, on May 27, 2023. Before death, the tortoises exhibited symptoms of inappetence and severe diarrhea, just shortly after the administration of 1% Ivermectin (Ivanor from Jubaili Animal Health Ltd) at the dose rate of 0.04 mg/kg. The medications were administered due to heavy tick infestation and diarrhea noticed by the client earlier. Shortly after the administration, three of the eight tortoises died and two died after a few hours as well.

Postmortem examination was performed through organ dissection with scissors and trump forceps to observe pathological changes in the internal organs. Histological tissue sectioning was also carried out on the liver samples and stained with Hematoxylin and Eosin (AppliChem, Darmstadt, Germany) was performed on 2 and 5 µm thick paraffin sections as well as a special stain named Trichome as described by Exbrayat [42] and observed under microscope at X100 and X400, respectively.

Two different blood samples were taken as described by Stuart et al. [43] from the three surviving tortoises with 1-inch 23–25 gauge syringes (Made by Delejet) as explained by Donald et al. [44]. The first one was transferred into heparinized tubes (Vacutainer, Becton Dick inson, Franklin Lakes, NJ 07417) for hematology screening. The second sample was split into two parts. One was centrifuged at 2,000 revolutions per minute for 15 minutes, and the plasma was submitted for biochemical analysis at the Clinical pathology unit. Hematology analyses included packed cell volume (PCV), heterophil, estimated total white blood cell count (WBCC), and hemoglobin concentration. The PCV was determined by microhematocrit centrifugation at 12,000 g for 5 minutes and blood smears were fixed in methanol and stained with Wright-Giemsa and estimation of total WBCC and *Borellia spp.* screening as explained by Larsson and Bergström [45]. While the remaining blood sample was used for microbial culture for *Escherichia coli* and *Salmonella enterica.*

Escherichia coli was detected through the technique explained by Abbas and Tuttle [5,46]. and *S. enterica* was also detected as described by Ferone et al. [47]. Evaluation of the three fecal samples collected from the surviving tortoises through the technique by Cringoli et al. [48]. This revealed at least 3 O*xyurid spp.* nematode ova and cysts per X10 (1+) field by direct smear and 10 *Oxyurid* nematode ova per X10 field (3+) by sodium nitrate flotation technique as explained by Cringoli et al. [48].

Results

Deceased tortoises

Postmortem examinations revealed significant findings among the deceased tortoises. Hemorrhagic lung tissues were evident in five of the tortoises, indicating potential respiratory issues. In addition, all of them exhibited fatty liver, suggestive of underlying metabolic or dietary concerns. Furthermore, four tortoises displayed signs of bladder inflammation, with three of them also presenting bladder stones, indicating urinary tract complications. Notably, ticks were found on all the tortoises, underscoring the prevalence of external parasites in their

Table 1. Post-mortem (gross) findings of the five deceased tortoises.

Organs	Lungs (Hem.)	Liver (Fatty)	Bladder (Infl.)	Bladder (Stones)	Skin (POT)	Kidney (Hem.)	Intestine (Perf. and Infl.)
Tortoises							
A	Present	Present	Present	Present	Present	Present	Present
B	Present	Present	Absent	Absent	Present	Absent	Absent
C	Present	Present	Present	Absent	Present	Present	Present
D	Present	Present	Present	Present	Present	Present	Present
E	Present	Present	Present	Present	Present	Present	Present
Total	5/5	5/5	4/5	3/5	5/5	4/5	4/5

Keys: Hem. = Hemorrhagic, Infl. = Inflamed/ Inflammation, POT= Presence of Ticks, Perf. = Perforation.

environment. Pin-point hemorrhagic kidneys were observed in all five tortoises, suggesting potential renal issues. Moreover, perforated and inflamed intestines were observed in all deceased tortoises, indicating gastrointestinal complications (Table 1). Histological staining revealed hepatic vacuolation in all deceased tortoises, further emphasizing the extent of pathological changes within their organs (Table 2).

Table 2. Liver histological findings of the five deceased tortoises.

Findings	Hepatic vacoulation
Tortoises	
A	Present
B	Present
C	Present
D	Present
E	Present

Surviving tortoises

The laboratory analyses, as depicted in Table 3, revealed noteworthy findings regarding the surviving tortoises. Their PCVs were all lower than the normal range, indicating potential anemia. In addition, there was a slight increase in heterophils and white cell counts among the tortoises, suggesting a mild inflammatory response. Two of the tortoises exhibited lower hemoglobin concentrations compared to the third tortoise, indicating varying degrees of blood oxygen-carrying capacity.

Furthermore, the results from microbial culture and Parasitology, outlined in Table 4, highlighted additional concerns. *Borrelia spp*. was detected in the blood smear, indicating a potential tick-borne infection. In the fecal sample, the presence of *Oxyuris spp*. eggs and cysts were observed, indicating gastrointestinal parasitism. Moreover, microbial culture identified *E. coli* and *S. enterica*, raising concerns about bacterial infections in the tortoises. These findings revealed the complex health challenges faced by the surviving tortoises and emphasize the need for comprehensive veterinary management.

Table 3. Blood picture from the surviving tortoises (Dilruksh et al. [50]).

Parameters	PCV (21.30%–29.0%)	Heterophils (1.52–4.30 × 10^9/l)	White cell count (4.33–9.80 × 10^9/l)	Hemoglobin (0.49–0.67 g/dl)
Tortoise				
A	21	5.32	9.84	0.57
B	18	5.71	9.93	0.31
C	20	5.21	9.22	0.45

Table 4. The outcome of the microbial culture and parasitic eggs conducted on the surviving tortoises.

Organism	*Escherichia coli*	*Salmonella enterica*	*Borellia spp*	*Oxyuris spp eggs*
Tortoises				
A	Present	Present	Present	Seen
B	Present	Present	Present	Seen
C	Present	Present	Present	Seen

Discussion

The Hermann's Tortoise, classified as "near threatened" by the International Union for Conservation of Nature [49], is a species from the family of Chelonians that holds immense ecological significance. However, despite its protected status, illegal collection from the wild for the pet trade remains a pressing concern [51]. Many of these tortoises, intended as pets, either suffer neglect or are seized by conservation authorities [52], underscoring the urgency of addressing conservation and welfare issues surrounding this species.

Apart from ticks, tortoises can be infested by various other ectoparasites. However, there is limited information available regarding the ectoparasites species affecting tortoises [53]. This scarcity call for further research the necessity for further research and studies in this area [53], highlighting the need to enhance our understanding of tortoise ectoparasitism. While tick-borne pathogens in mammals and birds have received substantial attention [54,55], the potential role of reptiles as reservoirs for zoonotic infections has been largely overlooked [8], particularly in regions like Nigeria. Consequently, pet tortoises may serve as reservoirs for tick-borne Zoonoses if proper management and husbandry practices are not instituted.

Post-mortem examinations conducted in this study unveiled a complex array of health issues, encompassing tick infestations, helminthosis, and Borelliosis, highlighting the multifaceted nature of the challenges faced by the tortoises. While the presence of *Oxyuris spp*. in the fecal sample may not immediately raise concern, as it's been documented as the predominant nematode in Hermann's tortoises [39], caution is warranted. Hallinger et al. [53] emphasize the prevalence of oxyurid infection in captive tortoises, noting that it often remains asymptomatic and may even serve a beneficial role in aiding digestion [39,56]. However, an excessive parasite burden can precipitate severe clinical manifestations, including anorexia, intestinal lacerations, inflammation, and perforation [53,57], as also observed in our study (Table 1).

More so, the treatment of nematodiasis in tortoises poses significant challenges due to the difficulties in administering medications orally and the potentially toxic effects of anthelmintics like Ivermectin. The use of Ivermectin, in particular, presents hurdles compounded by its prolonged gastrointestinal transit time, which can impede drug absorption [48]. This emphasized the complexities the complexities involved in managing parasitic infections in tortoises and highlights the importance of tailored treatment approaches to ensure optimal outcomes.

The observation of hepatic vacuolation in Figures 1 and 2 and fatty liver in Figure 3 raises concerns regarding potential intoxication or adverse drug reactions stemming from the dosage of Ivermectin administered during treatment, as highlighted by Cringoli et al. [48] also. This revealed the acute the acute and potentially life-threatening nature of Ivermectin usage in managing nematodiasis in Hermann's tortoises, a sentiment echoed in the findings of Teare and Bush [41] concerning red-footed tortoises.

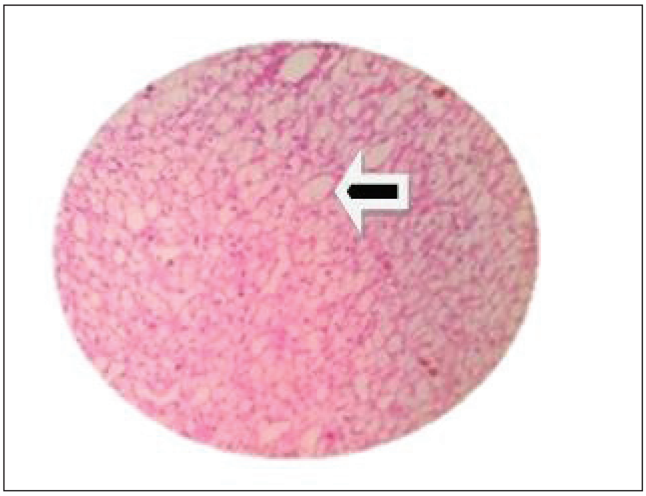

Figure 1. A photograph of the liver showing hepatic vacuolar degeneration (black arrow) with the use of Hematoxylin & Eosin stain (×100).

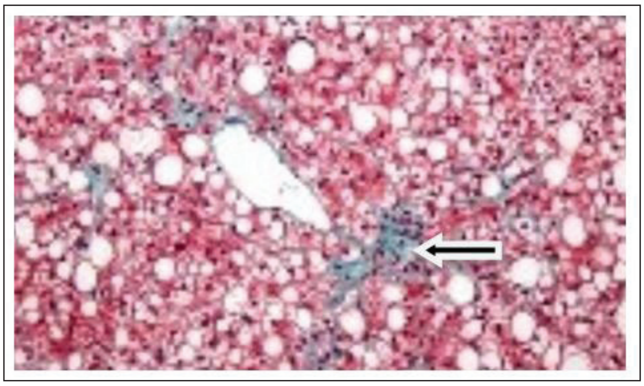

Figure 2. A photograph of the liver showing hepatic vacuolar degeneration (black arrow) with use of special stain (Trichrome ×400).

Also noted in our findings, was the presence of hemorrhagic lung (Fig. 4), kidney, and liver tissues, as well as general internal organs (Fig. 5), which indicates the likelihood of an acute mixed infection involving *E. coli, S. enterica*, and Borelliosis, as evidenced in Table 4. This further complicates the clinical picture, which also aligns with the observations of Hallinger et al. [53] and Pak Kan et al. [57] as well. These findings underscore the intricate nature of the health challenges faced by the tortoises and highlight the potential for fatal complications if left unaddressed.

Moreover, the identification of urinary calculi (Fig. 6) in the bladder revealed concerns regarding concerns regarding renal health in this species.

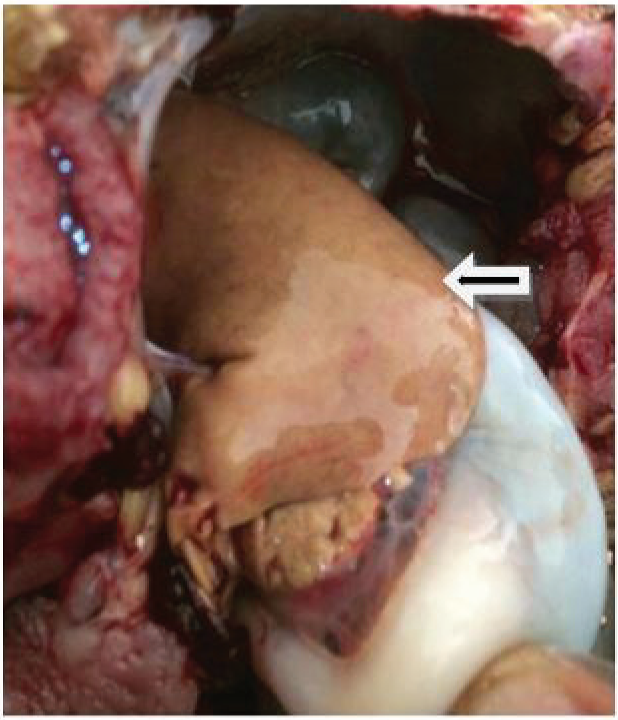

Figure 3. A photograph of liver showing fatty liver (black arrow) due to acute ivermectin toxicity.

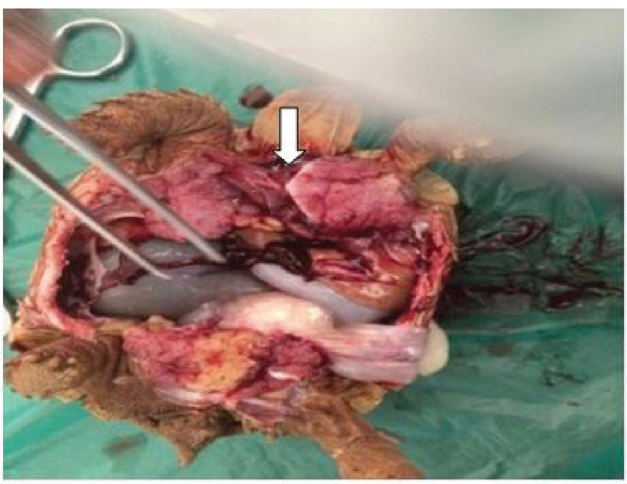

Figure 4. A photograph of the lung tissues (white arrow) showing haemorrhagic lesion.

Figure 6. A photograph of the stone (urolith) found in the bladder of the carcasses.

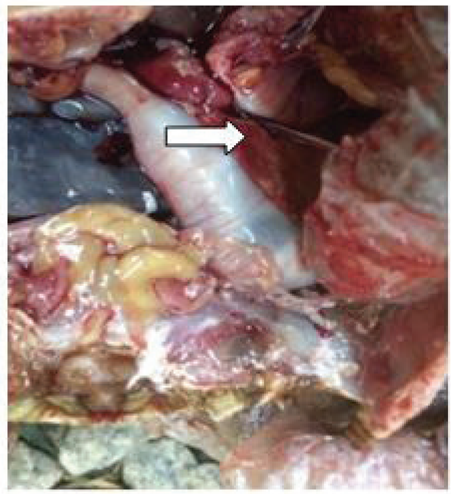

Figure 5. A photograph of the internal organs showing generalized hemorrhagic lesion(white arrow).

While urinary calculi are documented in reptiles, including chelonians, the precise etiology in tortoises remains unclear [58,59]. Comprised mainly of urate, these calculi may be influenced by dietary factors, emphasizing the critical role of nutrition in the care of captive tortoises [60].

The emergence of Ivermectin-induced toxicity in this study raises notable concerns, particularly given its association with the mortality of five out of the eight tortoises. Some veterinarians and herpetoculturists caution against the use of Ivermectin in chelonians, citing potential neurological complications and subsequent fatalities [40,41], a concern corroborated by our observations. Reports also document toxicity from Ivermectin in various chelonian species, although Hammern's Tortoise is not specifically mentioned, leading to progressive paralysis and ultimately death due to respiratory muscle paralysis [41]. This aligns with our findings as depicted in Table 3. Tortoises, notably the Leopard Tortoise (*Geochelone pardalis*), appear to be particularly susceptible to Ivermectin toxicity compared to other chelonians [40,41]. However, there remains a lack of significant studies evaluating the efficacy, dose-related effects, and toxicity of Ivermectin specifically in Hermann's Tortoises.

The findings from Table 3 revealed low PCV levels in all three tortoises, while an increase in heterophils in these tortoises also corresponds to the observations reported by Donald et al. [44]. This may suggest generalized inflammation and hemorrhagic organ involvement observed in the deceased tortoises, possibly due to underlying bacterial infections. In addition, the elevated WBCC in Table 3 could be attributed to the presence of *Oxyuris spp.* as noted in Table 4. Furthermore, the low hemoglobin concentration in two surviving tortoises might also be indicative of toxicity resulting from Ivermectin, consistent with findings reported by Yang et al. [61].

The findings from this analysis, along with insights from previous studies, underscore the critical importance of exercising caution and precision when administering medications to reptilian species, particularly with the use of Ivermectin. The inadvertent administration of potentially excessive doses of Ivermectin highlights the need for greater awareness and adherence to safe dosage guidelines specific to this species. While supportive care enabled three tortoises to survive, the underlying pathophysiology of Ivermectin toxicity in tortoises, especially the Hermann's species, necessitates thorough investigation to prevent similar incidents in the future. Understanding the mechanisms by which Ivermectin exerts its toxic effects can guide the development of more targeted and effective treatment strategies, ultimately safeguarding the well-being of reptile populations.

In light of these findings, it is crucial to adopt a comprehensive approach to diagnosis and management to ensure optimal outcomes for tortoises, including the Hermann's species. By thoroughly investigating the underlying causes of toxicity and addressing associated infections and complications, veterinary professionals can minimize the risk of adverse outcomes and enhance the overall prognosis for reptilian patients. In addition, ongoing monitoring and surveillance are essential to promptly detect and address emerging health issues in reptiles, thereby reducing the likelihood of similar incidents occurring in the future.

Conclusion

Ivermectin-induced toxicity represents a grave concern for Hermann's tortoises and other animals, particularly when dosages are administered without careful consideration. This emphasized the critical need to understand the importance of judicious medication administration and diligent monitoring within reptile husbandry practices. It is imperative that clinicians exercise extreme caution when prescribing antiparasitic drugs, particularly in the case of tortoises, and explore alternative treatment options whenever feasible to mitigate potential adverse effects on this species. Moreover, regular health assessments and parasite screenings must be integrated into the routine care of captive and pet tortoises to safeguard their well-being.

In addition to pharmacological considerations, proper husbandry practices play a pivotal role in preventing similar incidents within captive tortoise populations. Implementing deworming protocols, maintaining optimal environmental hygiene, and adhering to rigorous standards of care are paramount to ensuring the health and vitality of these magnificent creatures. By prioritizing conscientious medication administration, vigilant monitoring, and proactive health management strategies, we can collectively work towards safeguarding the welfare of Hermann's Tortoises and other reptilian species in captivity, thereby fostering a harmonious coexistence between humans and the natural world.

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Conflict of interest

No external funding was received for the preparation of this manuscript.

Author's contributions

All authors contributed equally to the conception, design, drafting, and revision of the manuscript. MH, DDI, and RAB conducted the *post-mortem* analysis and collected all samples for laboratory analysis. ORA processed all samples in the laboratory. ZIO edited, reviewed, and proofread the manuscript. ABH provided the manuscript write up, overarching supervision and guidance throughout the development of the main components of this work.

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