

Fatal amoebiasis in a group of Russian tortoises (*Testudo horsfieldii*)

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Case history and clinical management

To a private tortoise collection four 2-year-old Russian tortoises (*Testudo horsfieldii*) bred in captivity were added but kept separately in an outdoor quarantine pen. Twice within two weeks faecal samples were examined for parasites and were found to be negative. After four months three of the animals had died, two of which showed no preceding clinical disease. Only one animal had shown anorexia prior to death in spite of being treated with metronidazole for ten days. The fourth surviving animal additionally received paromomycine, marbofloxacin and physiological saline infusions, and showed no clinical signs of disease.

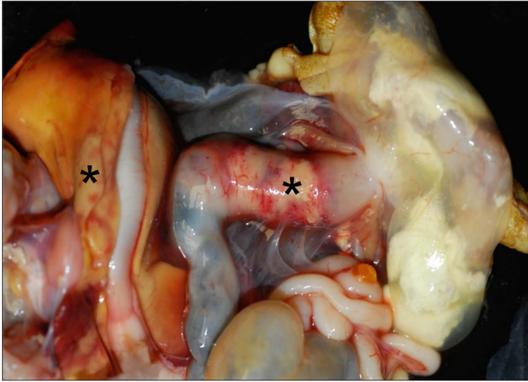


Fig. 1: Coelomic cavity: note beige discolouration of colonic and hepatic tissue (asterisks). (case #2)

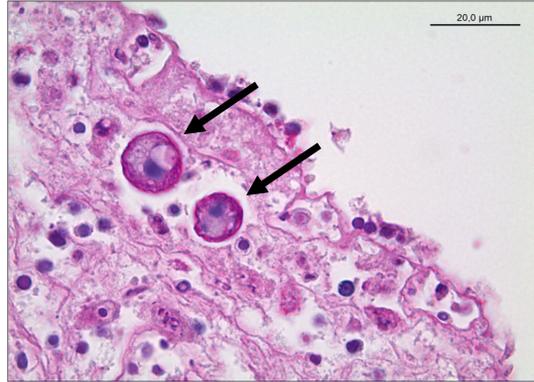


Fig. 2: Colon: two invasive amoebae (arrows). PAS reaction, 100 x obj. (case #2)

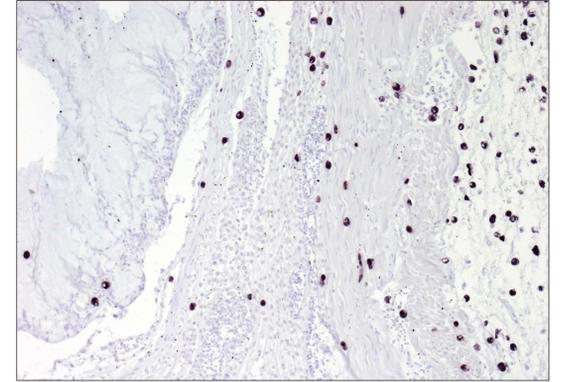


Fig. 3: Colon: ISH for *E. invadens* demonstrates numerous darkly stained protozoa throughout the colonic wall. ISH, 10 x obj. (case #2)

Pathologic and molecular examination

The three deceased animals were submitted for pathological examination. They showed ulcerative colitis (n=2) and/or multifocal hepatic necrosis (n=2, Fig. 1 & 4). Histologically, numerous amoeboid protozoa were detected within the necrotic lesions in macroscopically altered organs (Fig. 2 & 5). Especially in the diphtheritically inflamed colon the amoebae could be seen to invade all layers of the intestinal wall and also blood vessels suggesting haematological spread to the liver. This could be seen very clearly with chromogenic in-situ hybridization (ISH) specific for *Entamoeba invadens* (Fig. 3 & 6).



Fig. 4: Liver: multifocal beige and red discolouration compatible with hepatic necrosis. (case #1)

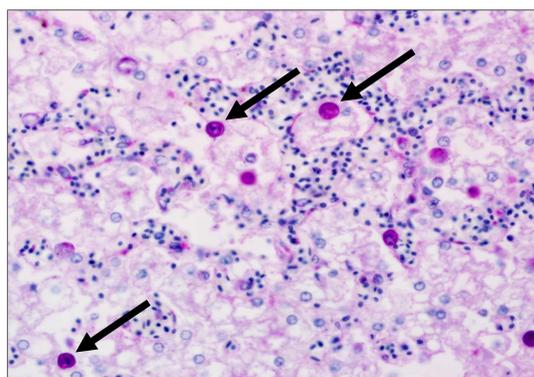


Fig. 5: Liver: multiple PAS positive, invasive amoebae (arrows) with dark chromidial bodies. PAS reaction, 40 x obj. (case #1)

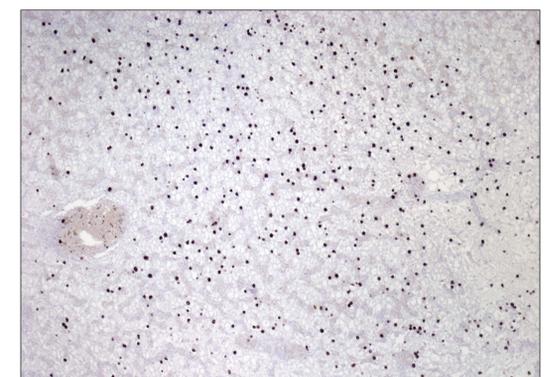


Fig. 6: Liver: ISH for *E. invadens*. Numerous protozoa invading hepatic tissue. ISH, 4 x obj. (case #1)

Discussion

Fatal *E. invadens* infections are well known in snakes, but are comparably rare in herbivorous reptiles, such as tortoises. In herbivorous animals the amoebae usually remain inside the intestinal lumen without causing tissue lesions. Recent transportation and stress are considered major factors for the development of disease in tortoises. Intermittent faecal shedding of cysts and/or the invasive behaviour of the amoebae might explain the negative coproscopic examination results. The animals' history, the incubation period, and the pathological lesions seen in the present cases are in accordance with previous reports, but clinical symptoms of unspecific nature were observed in one of the animals only. An ultimate antemortem diagnosis of *E. invadens* is difficult as the morphology of the mature cyst with four nuclei is similar to non- or slightly pathogenic species of *Entamoeba* (e.g. *E. insolita*, *E. terrapinae*, *E. ranarum*).

The therapeutic approach in the presumably infected but surviving fourth tortoise may well illustrate the need for a combination of compounds for successful treatment of amoebiasis in turtles. The inclusion of compounds being effective to extraintestinal stages via absorption across the intestinal mucosa (e.g. metronidazole) as well as compounds directly effective on the intestinal luminal stages (e.g. paromomycine) is required.