PCR-CONFIRMED *ENTAMOEBA INVADENS*-ASSOCIATED HEPATITIS AND COLITIS IN 3 CO-HOUSED HOME'S HINGEBACK TORTOISES (*KINIXYS HOMEANA*)

I.L. Payne, M.F. Stidworthy, S.M. Thornton and A.F. Rich

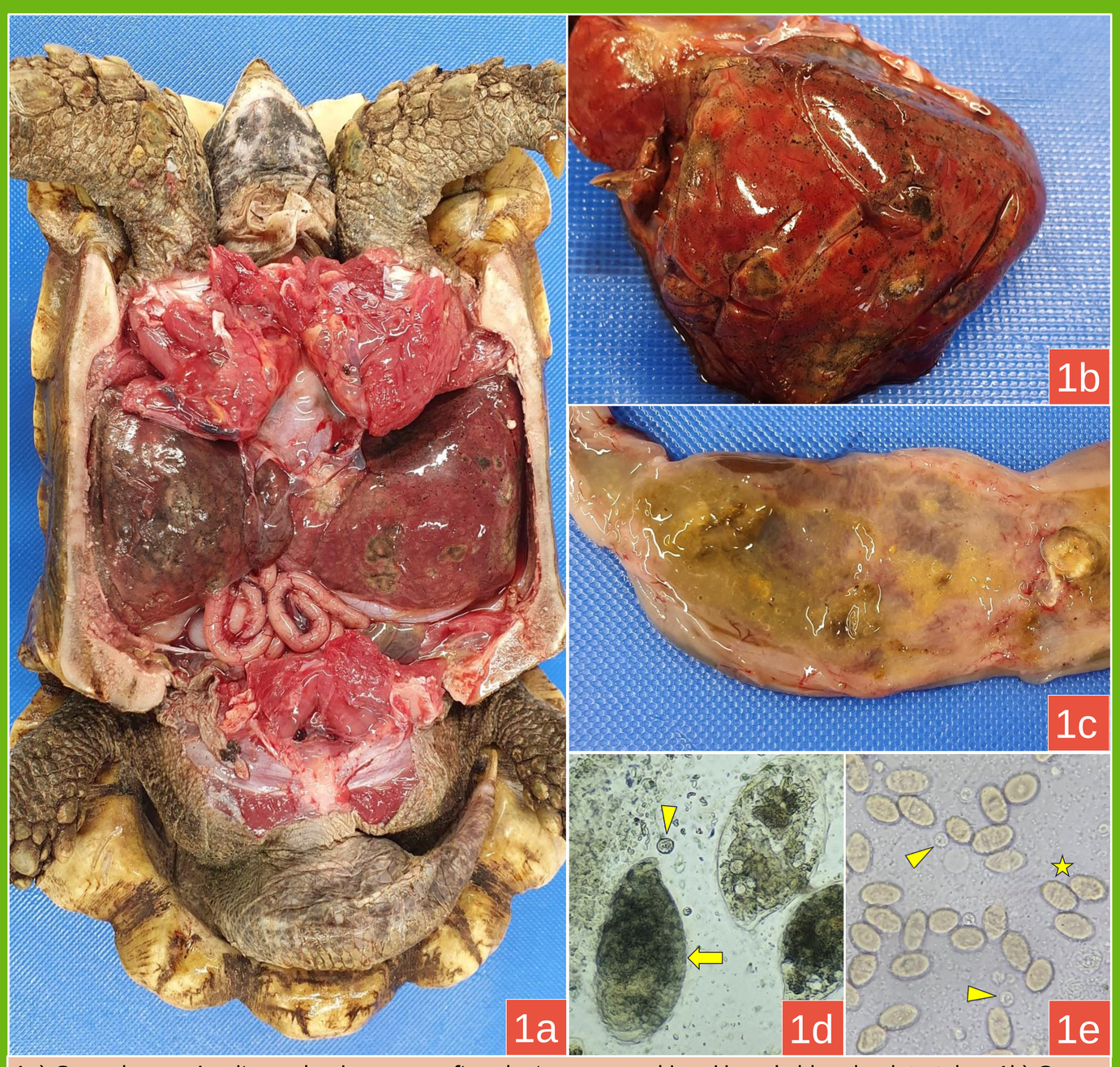
International Zoo Veterinary Group, Keighley, GB.

Introduction

Entamoeba invadens is an amoeboid protozoan known to be highly pathogenic in lizards and snakes. A single PCR-confirmed case is documented in a river turtle but reports of presumptive cases affecting terrestrial chelonians lack molecular confirmation by PCR testing. This presentation documents a rare outbreak of PCR-confirmed *Entamoeba invadens* in 3 co-housed terrestrial chelonians.

Materials & Methods

Three deceased Home's hingeback tortoises (*Kinixys homeana*) were submitted by a single zoological collection over a two-month period for full post-mortem examination.



1a) Gross image: In-situ coelomic organs after plastron removal in a Home's hingeback tortoise. 1b) Gross image: Liver with hepatomegaly, multifocal-to-coalescing hepatic necrosis and melanin pigmentation. 1c) Gross image: Cut-section of large intestine with catarrhal and erosive colitis showing submucosa oedema. 1d & 1e) Microscopy image: Direct wet prep of large intestinal contents, identifying large ciliate protozoa, consistent with *Balantidium coli* (arrow), smaller non-ciliate "*Entamoeba*-like" protozoa (arrowhead), and red blood cells (star).

Results

Primary gross lesions included:

1) Hepatomegaly with multifocal, 2-5mm diameter, pale-cream/tan, irregularly circular depressions rimmed by melanin pigment over the capsular surface and cut surfaces.
2) Catarrhal colitis with diffuse submucosal oedematous thickening, reddened mucosa, and moderate amounts of intraluminal mucoid fluid.

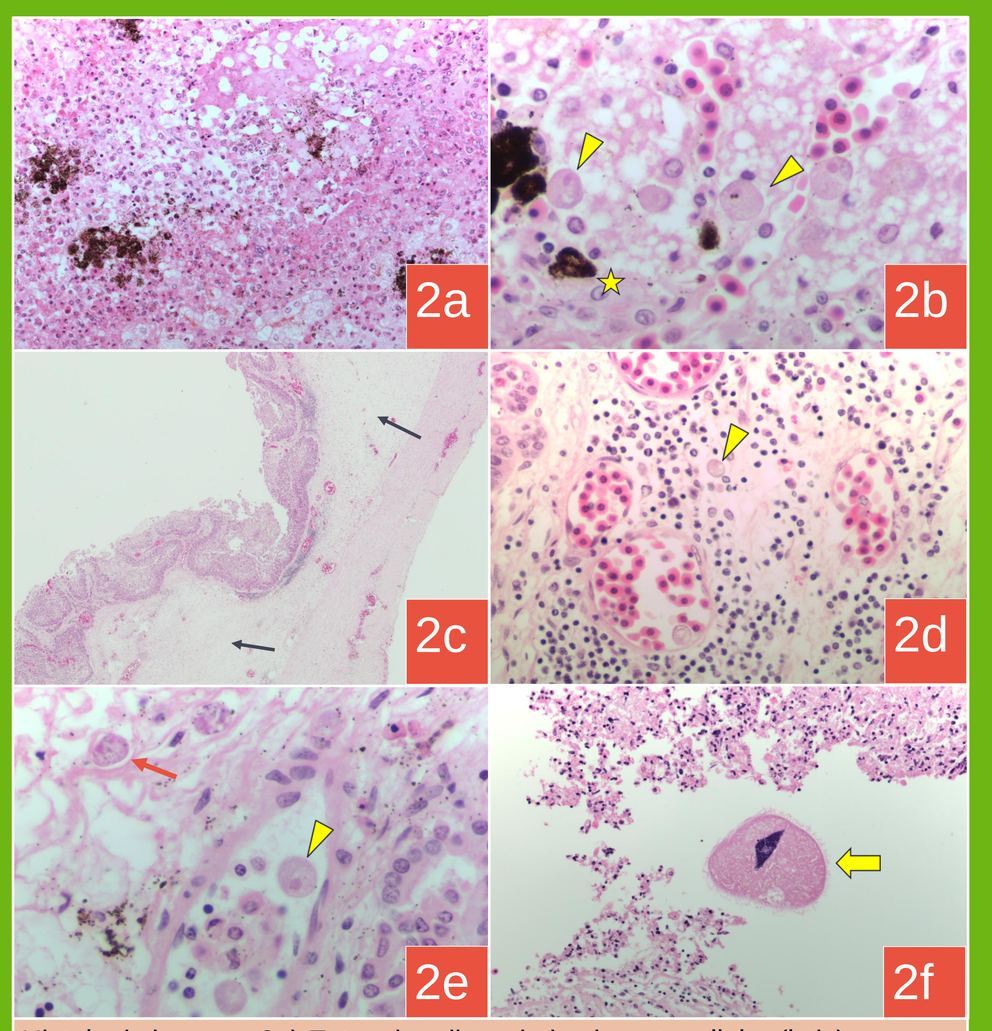
Direct microscopy of colonic content revealed moderate numbers of large ciliate protozoa (consistent with *Balantidium coli*) and low numbers of smaller non-ciliate "*Entamoeba*-like" protozoa.

Results

Main histological lesions included:

1) Marked, multifocal-to-coalescing, random, acute hepatic necrosis with intralesional protozoa (consistent with *Entamoeba* spp.) and occasional small bacillary bacterial colonies.
2) Marked, subacute, diffuse, erosive and heterophilic colitis with submucosa oedema and intralesional (*Entamoeba*-like) protozoa and mixed colonising bacteria.

Pooled liver samples were positive on PCR analysis for *Entamoeba* spp. and *Entamoeba invadens*.



Histologic images. 2a) Extensive dissociative hepatocellular (lytic) necrosis with melanomacrophage hyperplasia, liver (HE, 200x). 2b) Intralesional small non-ciliate "*Entamoeba*-like" protozoa (arrowhead) and melanomacrophages (star), liver (HE, 400x). 2c) Catarrhal and erosive colitis with severe diffuse submucosa oedema (arrow), large intestine (HE, 20x). 2d) Submucosal small non-ciliate "*Entamoeba*-like" protozoa (arrowhead), large intestine (HE, 400x). 2e) Intravascular (arrowhead) and intraparenchymal (arrow) small non-ciliate "*Entamoeba*-like" protozoa, liver (HE, 600x). 2f) Intraluminal large ciliate protozoa, consistent with *Balantidium coli* (arrow), large intestine (HE, 400x).

Conclusion

This PCR-confirmed outbreak of *Entamoeba invadens* substantiates past suspicions that this *Entamoeba* species can induce clinical disease in terrestrial chelonians and may be transmissible. Whilst the source of infection was unknown, co-housed reptiles or faecal contamination cannot be excluded.

References:

- Tortoise image (top right) extracted from: https://www.lllreptile.com/products/31610-homes-hingeback-tortoises (06/08/2023).
- Bradford CM, Denver MC, Cranfield MR. Development of a polymerase chain reaction test for *Entamoeba invadens*. Journal of Zoo and Wildlife Medicine. 2008;39(2): 201-207.
- Garcia G, Ramos F, Perez RG, et al. Molecular epidemiology and genetic diversity of *Entamoeba* species in a chelonian collection. Journal of Medical Microbiology. 2014;63(2): 271-283.
- Hollamby S, Murphy D, Schiller CA. An epizootic of amoebiasis in a mixed species collection of juvenile tortoises. Journal of Herpetological Medicine and Surgery. 2000;10(1): 9-15.