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Assessing Cryptosporidium presence in pet owned and introduced non-native freshwater turtles in the UK

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Research Article

Keywords: Invasive, Zoonotic, Chelonia - Pathogen

Posted Date: November 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11019885/v1

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Additional Declarations: No competing interests reported.

Abstract

Purpose

Introduced non-native species are recognised as a reservoir for zoonotic transmission, posing risks to both wildlife and humans. The presence of *Cryptosporidium* spp. (protozoan parasite) has not been previously studied in rehomed or feral pet freshwater turtles in the UK. These animals may pose risks to pet owners when carrying out husbandry activities and if released/escaped could further contribute to pathogen pollution in the wild.

Methods

Between 2021–2023, both pre-owned and introduced turtles were housed temporarily for a period of 14 days in separate holding containers. In total, 77 faecal samples were collected: turtles brought for rehoming by pet owners (n = 35) and non-native turtles removed from fresh waterbodies across England (n = 42). Faecal samples were initially tested using quick stick assays. The DNA was then extracted from samples, and both PCR and Sanger sequencing were carried out to identify *Cryptosporidium* presence.

Results

In total, 57 (74%) samples tested PCR positive for *Cryptosporidium*. Although there were a high number of positive PCR results, only 2 (5%) tested positive from further Sanger sequencing. These were two separately rehomed pet *Pseudemys* spp. (cooter turtles), *Cryptosporidium ryanae* and *C. parvum*. *C. ryanae*, is a bovine intestinal parasite of low pathogenicity rarely causing critical illness therefore the individual sampled is assumed an asymptomatic mechanical vector. However, *C. parvum* was identified in a pet turtle brought for rehoming. *C. parvum* being a highly pathogenic zoonotic parasite causing severe to fatal clinical outcomes in young and immunocompromised. This may pose a risk to pet owners and pathogen pollution as a result of release into the wild.

Conclusion

Although the parasite was found present, the results imply a low risk of transmission. Further investigation is suggested to understand the possible impacts on native wildlife and humans as a result of introduced pet turtles.

Introduction

Cryptosporidium is a protozoan parasite often transmitted via the faecal-oral route through food and water sources, with the potential risk of the disease ‘cryptosporidiosis’ [1, 2]. It is primarily identified from mammals [3–12] but can also be found in other living host organisms, such as reptiles, birds and fish

[13–23]. The parasite targets epithelial cells of the small intestine where pathogenicity causes cell damage and microvilli alteration causing harm [24]. Host-adapted species without clinical disease may be at risk if immunocompromised or young and can pose zoonotic transmission to other species. In these instances, succumbing to cryptosporidiosis can result in severe dehydration (watery diarrhoea), vomiting, weight loss and mortality, if unable to combat symptoms [1]. Hosts can shed oocysts for several weeks into the environment following the illness [25, 26].

Studies on captive tortoises, have shown *Cryptosporidium* transmission to other reptiles including snakes and lizards [20, 27–29]. Broader research of the parasite in reptiles, such as *C. serpentis* (snake), have shown presence in the gut mucosa causing gastritis and enteritis [30]. Swelling can occur in the mid region of the body with food regurgitation due to mononuclear cell inflammation (thickening) in the smaller intestine [29]. This can result in extreme weight loss (emaciation) and diarrhoea. *C. serpentis* has been detected primarily in captive snakes but also in wild individuals [30]. There are limited *Cryptosporidium* studies in freshwater turtles [13, 14, 19, 29]. Rzeźutka et al. (2020) [14], aimed to understand *Cryptosporidium* presence in introduced pet turtles in freshwater locations in Poland. In total, 104 individuals were removed and sampled including: red-eared sliders *Trachemys scripta elegans*, yellow-bellied sliders *T. s. scripta*, Cumberland sliders *T. s. troostii* and false map turtles *Graptemys pseudogeographica*. Screening results showed one sample testing positive for *C. parvum* (IAS listed species: *T. s. elegans*). Although a highly pathogenic zoonotic strain, freshwater turtles and other reptiles often appear to be a mechanical carrier as opposed to being symptomatic and suffering from the disease [31].

There has been little investigation into the potential spread of parasites from introduced pet turtles to other species in both wild and captive environments. One study by Pino-Vera et al. (2025) [32], screened non-native freshwater turtles in Tenerife and Gran Canaria (Spain) identifying multiple pathogenic bacteria posing risks to humans and wildlife. With EU regulations in place to restrict sales, breeding and keeping of Invasive (Non-native) Alien Species (IAS) [33], such as the *Trachemys scripta* ssp., the act of releasing pet turtles into the wild, continues to be an issue. The aim of the study was to test for *Cryptosporidium* presence in feral and pet freshwater turtles to identify potential risks: 1) of pathogen pollution from released or escaped individuals into the wild 2) to pet owners and rescue/rehoming facilities caring for turtles.

Materials and methods

Sample collection

Between February 2021 and December 2023, 77 freshwater turtles were sampled as part of a wider body of research on introduced non-native species (Turtle Tally UK). Individuals sampled comprised of: *Chelydra serpentina* (Common snapping turtle), *Graptemys* spp. (map turtles), *Sternotherus odoratus* (Common musk turtle), *Pelodiscus sinensis* (Chinese softshell turtle), *T. s. elegans* (Red-eared slider) and *T. s. scripta* (Yellow-bellied slider). During this period, feral turtles were caught safely and humanely (by

hand/netting) from freshwater bodies across England having been reported and asking for them to be removed. All individuals were health assessed on admission to the National Centre for Reptile Welfare (NCRW) and temporarily housed separately in clean plastic holding containers (~ 70 cm x 40 cm x 40 cm) for up to 14 days. The containers were filled to a water depth of ~ 10 cm and, refreshed daily or when soiled. Food was not provided during this period to minimise the introduction of any further pathogens or contaminants. Each animal was monitored and checked daily for faecal excretions. Faeces were then collected in sterile pots, labelled and stored at -20^{o}C.

Immunochromatographic Quick Test Kit Assay

RIDA ®QUICK Cryptosporidium (N1203) test kits were initially used tried to assess efficacy compared to further DNA testing, as a potential quick test method to identify *C. parvum* presence from stool samples. Test kit instructions were followed, and all samples were tested and recorded.

DNA Extraction. PCR and DNA Sequencing

Stored samples were thawed for DNA extractions using ThermoFisher™ PureLink™ Microbiome DNA Purification Kit (Host DNA From Stool Samples) (catalogue number: A29790) in the University of Kent, School of BioSciences lab. No invasive techniques were used to collect samples. Nested PCR techniques were used to amplify the region identifying *Cryptosporidium* spp. of the (SSU) 18S rRNA gene. Positive and negative controls were used in the first and subsequent reactions. The primers used included: forward CRY_SSU_F1 5'AACCTGGTTGATCCTGCCAGTAGTC-3' and reverse primer CRY_SSU_R1 5'AACCTGGTTGATCCTGCCAGTAGTC-3' in the first PCR reaction. The first PCR reaction run process (24 cycles), initial denaturation at 94^{o}C for 2 min then denaturation at 94^{o}C for 50 s, annealing at 53^{o}C for 50 s then elongation at 72^{o}C for 1 min. Then final elongation at 72^{o}C for 10 mins. In the second PCR reaction the following primers were used: CRY_SSU_F2 5'-CAGTTATAGTTTACTTGATAATC-3' and CRY_SSU_R2 5'-CCTGCTTTAAGCACTCTAATTTTC-3'. The second PCR reaction (30 cycles) initial denaturation at 94^{o}C for 2 mins, denaturation at 94^{o}C for 50 s, then annealing at 56^{o}C for 30 s and elongation at 72^{o}C for 1 min then 72^{o}C for 10 mins. DNA concentration was determined using a BioDrop machine. The assay produced was evaluated on 2% agarose gel with ethidium bromide added. Visualisation was carried out using gel electrophoresis and identification under UV light. Positive indicators were carefully removed, and the amplified product was extracted from the gel using the Gene Jet Gel Extraction Kit (Thermo Fisher Scientific, CA, USA) as per manufacturer's instructions. Purified samples were sent for Sanger sequencing at Eurofins Genomics (Wolverhampton, UK). Results were analysed using the chromatograms and sequences were entered into the Basic Local Alignment Search Tool (BLASTn) to compare against the GenBank nucleotide database.

Results

In total, 77 freshwater turtles were screened for *Cryptosporidium*. Of these, 35 were categorised as wild (W) individuals and 42 were rehomed (R) individuals. No wild caught turtles tested positive for *Cryptosporidium* through sequencing, only one tested positive using the RIDA ®QUICK test sticks. All test sticks produced a negative result otherwise. Out of 77 samples, 57 (74%) tested PCR positive for *Cryptosporidium*. These positive samples (28 wild/29 rehomed) were sent for Sanger sequencing to further identify species. Although PCR positive results were high, further specificity highlighted only two samples. Twenty-eight samples produced no hits therefore could not be used in BLASTn analysis. Several samples had shorter Contiguous Read Length (CRL) from the sequencing yields, the range across the sample group being between 100 and 735bp (3 samples > 630bp). Results showed two individuals testing positive, one showed *C. parvum* and another *C. ryanae*, both rehomed captive *Pseudemys* spp. (see Table 1.). The two positive samples were sent for Gp60 subtyping, but these runs were unsuccessful.

Table 1
Test positive results, percentages as to total number in category, not overall sample number.

Sample group	***n* Total**	**RIDA ®QUICK test +**	***n* PCR +**	***n* Sanger sequence +**
Rehomed individuals (R)	35	0	29 (82%)	2 (6%)
Wild individuals (W)	42	1	28 (67%)	0

Discussion

Results from this study show *Cryptosporidium* presence in two rehomed turtles, both *Pseudemys* spp. (ED171, ED185). These cooter turtles are more commonly kept particularly following the EU IAS regulations prohibiting the sale of all *Trachemys scripta* ssp. in 2016. The faecal samples tested positive through Sanger sequencing identifying two subspecies, *C. parvum* and *C. ryanae*. The individual (ED185) testing positive for *C. ryanae* in this study was a captive juvenile male, weighing 185g, with no injuries although signs of hyperaemia were noted (indicator of septicaemia). This strain is host specific to bovine species, commonly found in post-weaned calves. There was no indication the turtle was housed outside therefore it is unclear where this may have originated from. In a study by Rzeżutka et al. (2020) [14], the turtles were wild caught therefore this strain could be linked to cattle presence near and in waterbodies. *C. ryanae* presence may have been identified due to water or food source contamination in the captive environment. Due to host specificity to cattle, freshwater turtles are not considered true hosts (an asymptomatic carrier) and it is therefore not considered a risk to humans. Sample ED171, was tested twice and both were positive for *C. parvum* strain (highly pathogenic and zoonotic). This individual was an adult male, brought in as part of an eviction process and weighing 1.33kg. A visual health assessment was carried out. The carapace showed minor deformation, with extensive pitting (indicative of previous ulcerative shell disease) and scute retention. Scarring was present on the base of each hind foot from prior healed wounds and there was inflammation of both ocular areas. This individual could pose a risk to pet owners if hygiene and cleaning practices are not efficiently carried out. Owners should be washing hands regularly and/or wearing gloves following handling and husbandry, cleaning

contaminated items with boiling water or steaming methods [34, 35]. There are no antiprotozoal vaccines or highly effective antiparasitic treatments that prevent or cure the disease therefore infected individuals rely on a stronger immune response to combat infections [26, 27, 29, 36]

Environmental conditions are a consideration regarding persistence in waterbodies. *Cryptosporidium* oocysts are resilient in moderate temperature ranges but are rapidly inactivated in extreme climates such as higher temperatures (55°C +) or extreme cold (between − 22°C and − 70°C) [37–39]. With a more temperate climate in England, oocysts are more likely to persist i.e. 6 months at 20°C [40, 41]. With freshwater turtles spending a large proportion of time in water, the intermittent shedding of oocysts, over a prolonged period, via defaecation in the surrounding environment pose a transmission risk to other species present [18]. The strain *C. ryanae* would not pose a risk to humans but if released into waterbodies where cattle are present, this could pose a transmission risk. Due to the short 14-day holding period, some individuals did not produce faecal samples so could not be tested. They may have passed faeces prior to collection or may not have been feeding during the period prior to removal or rehoming (food availability, seasonality and brumation). It is suggested that the timeframe be extended to allow for defecation during temporary housing, guided by animal welfare considerations. Pooling samples over a period may also provide a better review of the parasite presence due to sporadic shedding of oocysts. Studies, such as Robinson et al. (2010) [42], created shedding profiles for small mammals showing intermittent shedding past the 14-day period. Consideration of this is particularly relevant in a rescue and rehoming setting where animals are frequently admitted, housed with other species (zoonotic transmission potential) and husbandry duties carried out by humans. Quick test kit assays were used as part of the investigation to assess whether these could be used by pet owners and/or rescue/rehoming centres as a quick and cost-effective way to assess *Cryptosporidium* presence. These did not prove effective yet potential applications for quick tests in these environments could have positive implications.

Although, the infection rate is determined as low, the risk of *Cryptosporidium* parasite being introduced to natural water sources via pet turtle introductions is a consideration. Further studies on *Cryptosporidium* and pathogenic bacteria would be advantageous, better informing human-animal interactions, conservation of native species and mitigation strategies of non-native invasives.

Declarations

Acknowledgements

Thank you to the NCRW for their support in the research, the volunteers assisting in removals and park reserves staff for access and permissions. Thank you to Dr David Roberts, alongside co-authors RG and AT, for supervisory support during the project.

Author contributions

SS, RG and AT conceived and designed the study. SS conducted fieldwork, sample collection, genetic and data analysis and wrote the manuscript paper. KP carried out sequencing of samples. All authors have read, reviewed and approved the final manuscript.

Ethical approval

Ethical approval was gained from the Ethics Committee of the School of Conservation and Anthropology, University of Kent Ethics (Application no. 149, Ethics ID: 20221655230373149).

Conflict of interest The authors declare that there has been no conflict of interest.

Consent to publish Consent was gained from the NCRW to publish data.

Funding UK Research and Innovation (UKRI) provided 'Further Action to Support Students Funding' for travel to remove animals from waterbodies in the UK.

Financial interests The authors have no relevant financial or non-financial interests to disclose.

Data availability All data collated has been included in the paper.

Animal use related to the NC3Rs

Replacement: No replacement could be used in this case. **Reduction:** Sample size minimal due to availability during study period. **Refinement:** All welfare requirements considered for process of removal and sample collection.

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