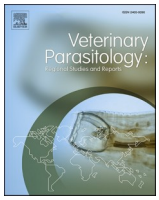
Veterinary Parasitology: Regional Studies and Reports 56 (2024) 101129

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr



Short Communication

Trypanosoma cruzi infection in American black bears (*Ursus americanus*): A case report in a cub from California and serologic survey for exposure in wild black bears from several states

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ARTICLE INFO

Keywords:
Bears
Chagas disease
Trypanosoma cruzi
Ursidae
Zoonotic diseases

ABSTRACT

Trypanosoma cruzi is an important cause of disease and death in humans and dogs, and although wildlife infections are common, less is known about disease manifestations. A 12-week-old male American black bear (*Ursus americanus*) cub with mild lethargy and anorexia presented to a wildlife rehabilitation center in Lake Tahoe, California. The cub continued to become increasingly weak and showed decreasing interest in play and other activities. The cub was anemic and had increased γ-glutamyltransferase (GGT) liver enzymes. A large number of trypanosomes were noted on a thin blood smear. *Trypanosoma cruzi* was isolated in culture from a subsequent blood collection. Proliferative bony lesions were noted on radiographs, but this finding was considered unrelated to the *T. cruzi* infection. The number of parasites observed in thin blood smears dramatically dropped over time, but it remained PCR positive until at least nine months. The cub continued to gain weight and became increasingly active. Serum samples from the cub were positive with three different serologic assays (IFA, ELISA, and ICT). The bear was not treated because of the decreasing parasitemia and the improvement in activity and appetite. Although the bear could not be released due to issues unrelated to *T. cruzi*, it remains healthy in a captive facility. Sequence analysis of the DHFR-TS and COII-ND1 gene sequences confirmed the bear was infected with DTC TcIV. Following the detection of this clinical case, a serologic survey was conducted to determine the prevalence of *T. cruzi* exposure of black bears in California, North Carolina, and Pennsylvania. Because no serologic assay has been validated for use in bears, three different assays were used. Marked differences in apparent seroprevalence range from 1% (requiring all three assays to be positive) to ~20.7% (requiring only one assay to be positive). Black bears are naturally exposed to *T. cruzi* across the United States. Future studies using PCR testing of tissues or blood would be needed to better understand the prevalence

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https://doi.org/10.1016/j.vprsr.2024.101129
Received 23 June 2024; Received in revised form 7 September 2024; Accepted 26 September 2024
Available online 28 September 2024

of *T. cruzi* in wild black bears, lineages most commonly associated with infection, and if *T. cruzi* represents a health threat to bears.

1. Introduction

Chagas disease, caused by the kinetoplastid protozoan parasite *Trypanosoma cruzi* (Kinetoplastea: Trypanosomatida), is considered a leading neglected tropical disease in humans and it also can cause disease in a wide range of mammals, including ursids (Hamer and Saunders, 2002; Hochberg and Montgomery, 2023). The parasite is endemic from the southern United States to southern South America, but most human cases occur in Central and South America (Hochberg and Montgomery, 2023). Clinical disease can occur in two phases, acute and chronic. The acute phase can last about two months and there are typically no symptoms or they are mild, such as fever, lymphadenopathy, anemia, hepatosplenomegaly. However, some individuals may develop severe or fatal myocarditis or encephalitis. Chronic disease may not develop for years and is characterized as nondetectable parasitemias and it may include cardiac disease or gastrointestinal tract involvement (e.g., megaesophagus or megacolon) (Bern et al., 2011; Mills, 2020). The vectors of *T. cruzi* are triatomine bugs (Hemiptera: Reduviidae), often referred to as kissing bugs or chinches, and transmission is through exposure to the feces of infected vectors (Martín-Escolano et al., 2022).

Infection prevalence in wildlife in the United States with *T. cruzi* is highly variable, but prevalence can be high in some species such as raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*), woodrats (*Neotoma* spp.), and striped skunks (*Mephitis mephitis*) (Yabsley and Noblet, 2002; Roellig et al., 2009; Brown et al., 2010; Bern et al., 2011; Charles et al., 2013; Busselman and Hamer, 2022). Mild lymphoplasmacytic myocarditis was observed in 21% of infected mesocarnivores (Curtis-Robles et al., 2016; Hodo et al., 2020), but disease outcomes among infected wildlife are rarely determined, owing to challenges in longitudinal surveillance of individual wild animals as well as biases in which animals can be sampled by wildlife researchers or hunters. The triatomine vectors are broadly distributed in the southern United States with recent novel detections as far north as Delaware, Illinois, and Nebraska (Busselman and Hamer, 2022; Santos et al., 2024; Peterson et al., 2024).

American black bears (*Ursus americanus*) are the most common and widely distributed species of bears in North America, ranging from Alaska to Mexico. Threats to black bears primarily include habitat loss and human-bear conflicts, and although the disease has not historically been considered a major threat to black bears, sarcoptic mange (caused by *Sarcoptes scabiei*) has become an emerging concern in the eastern United States highlighting the need for pathogen surveillance and mortality investigation (Niedringhaus et al., 2019). In 2022, an orphaned black bear cub in California presented to a rehabilitation center with progressive weakness and lethargy. Large numbers of trypomastigotes were noted on a blood smear and *T. cruzi* was subsequently confirmed by culture and molecular characterization. After a diagnosis of *T. cruzi* was confirmed, we conducted a serologic survey of black bears from several states to determine distribution and prevalence of exposure.

2. Case report and serologic survey

2.1. Case description of a black bear cub

In late April 2022, a 12-week-old male American black bear cub presented to a wildlife rehabilitation center in Lake Tahoe, California after being found on the Tule River Nation Reservation and attempts to locate the sow failed. The cub presented with mild lethargy and anorexia and weighed 2.3 kg. The cub continued to become increasingly weak and showed decreasing interest in play and other activities. On April 22, 2022, blood samples were taken and submitted for a complete blood count (CBC) and blood chemistry analysis to IDEXX Laboratories (Westbrook, Maine). The cub was anemic with a hematocrit of 21.3% (reference value ~45%) and a red blood cell (RBC) value of 4.82 M/μL (reference 6.75) (Schroeder, 1987). Additionally, the cub had increased γ-glutamyltransferase (GGT) liver enzyme (420 U/L, reference value 12.7) (Storm et al., 1988). Also, there was a left shift of neutrophils and numerous round 4–7 um structures, each containing small, centralized nuclei and short flagella, were observed on stained blood smears (Fig. 1a).

On May 3, 2022, additional blood samples were collected and sent to the Southeastern Cooperative Wildlife Disease Study (SCWDS, University of Georgia, Athens, GA USA) for further analysis. At the time of blood collection, blood smears were prepared and fixed in methanol. Smears were stained with Diff-Quik. Although an accurate parasitemia cannot be calculated without a known volume of blood for extra-cellular parasites, a standard method of reading smears was developed to allow comparison of parasite numbers between different bleeding dates. Fields were examined until ~50,000 erythrocytes were observed; a total of 286 trypomastigotes characteristic of *T. cruzi* were observed (Fig. 1b). An EDTA preserved whole blood was co-cultured with DH-82 canine macrophages as described and *T. cruzi* trypomastigotes were observed within six days (Supplemental File Video) (Hall et al., 2007; Patel et al., 2012; Yabsley et al., 2004).

Approximately 14 days after admission, the cub weighed 2.59 kg and showed signs of recovering energy and increasing interest in eating. On May 31, 2022, radiographs and ultrasound diagnostics were performed to assess cardiac health. Although no cardiac abnormalities were observed, radiographs revealed proliferative bony lesions in the humeri and femurs (Fig. 2). Follow-up radiographs were taken three months following intake, and these lesions showed signs of resolving.

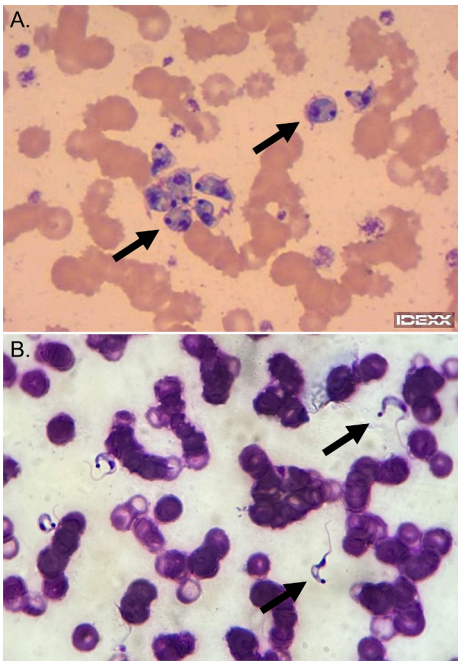

Fig. 1. Blood smears from a black bear (*Ursus americanus*) cub from California. A. Blood smear produced by IDEXX Laboratories showing numerous round structures interpreted as trypanosomes (arrows). B. Characteristic trypomastigotes of *Trypanosoma cruzi* (arrows) in a blood smear made with freshly collected blood.

At ~1 month following admission (June 2), a blood sample was collected and the parasitemia had dropped from 286 trypomastigotes to only a single parasite observed in fields containing 54,744 erythrocytes. The cub continued to gain weight and became increasingly active. Two months after admission (July 18), the cub was successfully integrated into an enclosure with another black bear cub. The cub was reported to play, climb, wrestle, and otherwise engage in activity without showing signs of struggle or infirmity. At this point, there was no detectable parasitemia in fields containing 59,592 erythrocytes (Table 1). However, the blood sample was PCR positive (see below for methods). A final blood sample was taken on January 23, 2023, and there was no detectable parasitemia and the sample was only weakly PCR positive for one PCR target (Table 1). Serum samples from the cub were tested with the three serologic assays described below and they were positive with all three assays (Table 1). A decision to not treat was made because of the decreasing parasitemia and the improvement in activity and appetite. To the date of this article (August 2024), the bear remains healthy, but because the bear was determined to be non-releasable (unrelated to *T. cruzi* infection), it was placed in a captive facility.

2.2. PCR detection and genotyping of T. cruzi from black bear cub

To determine if blood samples were PCR positive and to determine the genotype of *T. cruzi* from the black bear cub, DNA was extracted from whole blood samples and the cultured isolate. Detection of *T. cruzi* in blood samples was conducted by amplification of the D7 divergent domain of the 24Sα rRNA was conducted using primers D71 and D72 as described (Table 2).

Currently, *T. cruzi* is divided into at least seven lineages termed Discrete Typing Units (DTUs) TcI-TcVI and a bat-specific DTU Tcbat and these DTUs can be determined by sequence analysis of several different gene targets (Zingales et al., 2012). To determine the DTU present, partial sequences of the dihydrofolate reductase-thymidylate synthase (DHFR-TS) and mitochondrial cytochrome oxidase subunit II and NADH dehydrogenase subunit I (COII-ND1) genes were amplified using primers DH1S and DH3A and ND1.3A and COII.2A), respectively, as described (Table 2). Amplicons were visualized in a 1.5% agarose gel stained with GelRed (Biotium, Fremont, California). Amplicons were purified from the gels using a Qiagen Gel Extraction kit (Qiagen Germantown, Maryland) and submitted for bidirectional Sanger sequencing at Azenta Life Sciences (South Plainfield, Maryland). Sequences were analyzed using Geneious (Biomatters Limited, Auckland, New Zealand) and consensus sequences were compared with other *T. cruzi* sequences via nucleotide basic local alignment search tool (BLAST) in GenBank.

The 24Sα rRNA amplicon was ~130 bp in size which is consistent with TcIV DTU samples from North America (Brisse et al., 2001). The DHFR-TS sequence (1413 bp) was 100% identical to numerous TcIV DTU sequences from dogs, raccoons, Virginia opossums and *Triatoma sanguisuga* from the eastern USA (AF359010, GU212871, GU212873-GU212874, GU212884- GU212885, GU212892–994, GU212899, GU212901) (Machado and Ayala, 2001; Roellig et al., 2013). The COII-ND1 sequence (1171 bp) was also 100% identical to TcIV DTU sequences from a *Triatoma protracta* from California (KR135423, Shender et al., 2016), a *Triatoma gerstaeckeri* and two *T. sanguisuga* from Texas (MF670302, MF670303, MF670337; Flores-López et al., 2022) and numerous samples from the eastern US from dogs, raccoons, opossums, and *T. sanguisga* (HQ604892, HQ604909, AF359010, GU212889, GU212894, GU212873, GU212885, GU212888, GU212899, GU212901) (Machado and Ayala, 2001; Lewis et al., 2011; Roellig et al., 2013).

2.3. Serologic survey for T. cruzi in free-ranging black bears

2.3.1. Samples

Serum samples were previously collected from presumed healthy black bears from California ($n = 110$, 1978–2020), North Carolina ($n = 44$, 2019–2022), and Pennsylvania (n = 44, 2017–2018) during research trapping efforts or at hunter check stations. Samples were held at —20C and sent to SCWDS for testing. No animals were captured or sampled specifically for this study. However, acquisition of samples for diagnostic testing was reviewed and approved by UGA's Institutional Animal Care and Use Committee (IACUC) (A2018 02–010, A2020 11–010) and North Carolina State University's IACUC (16–263-O). When available, demographic information was obtained, but not all data were available for all samples.

2.3.2. Serologic assays

Serum samples were tested using three different serologic assays. The IFA assay was performed at the Texas A&M Veterinary Medical Diagnostic Laboratory using dilutions of 1:20 to 1:1280 and anti-canine immunoglobulin G (IgG) as the secondary antibody. The Chagas Detect™ Immunochromatographic test (ICT) was performed according to the manufacturer's protocol (InBios, Seattle, Washington); this rapid test is validated for use in humans. The enzyme-linked immunosorbent assay (ELISA) used was the Chagas IgG ELISA Kit from Alpha Diagnostic

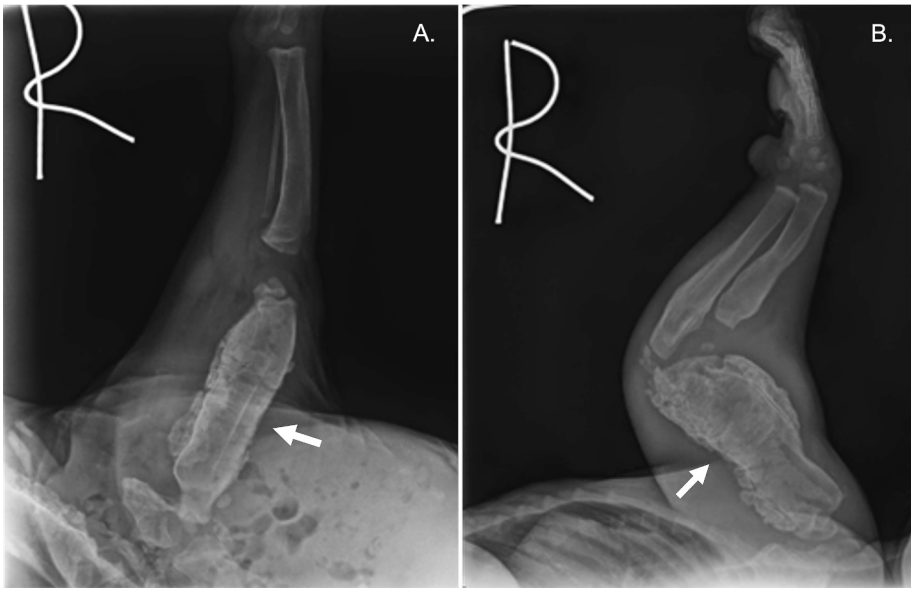

Fig. 2. Radiographs showing bony proliferative lesions (arrows) of the femurs (A) and humeri (B) in a black bear (*Ursus americanus*) cub from California.

Table 1
Summary of diagnostic results for a black bear cub (*Ursus americanus*) from California infected with *Trypanosoma cruzi*.

Date	Time from admission on April 21, 2022	No. of parasites observed in ~50,000 erythrocytes	PCR results on whole blood			Serology results		
			24Sα rRNA	DHFR-TS	COII-NDA	ELISA	ICT	IFA (titer)
April 22, 2022	1 day	Numerous*	N.D.**	N.D.	N.D.	N.D.	N.D.	N.D.
May 3, 2022	14 days	286	+	+	+	Positive	N.D.	N.D.
June 2, 2022	40 days	1	+	+	+	Positive	N.D.	N.D.
July 18, 2022	86 days	0	+	+	+	Positive	N.D.	N.D.
January 23, 2023	~9 months	0	−	+ (weak)	−	Positive	Positive	Positive (1:20)

* sample submitted to IDEXX Laboratories so a parasitemia was not calculated but parasites were readily observed in every field (Fig. 1a).
** N.D., not done, ELISA = Enzyme linked immunosorbent assay, ICT = Immunochromatographic test and IFA = indirect immunofluorescent antibody assay.

Table 2
Primers used to detect and genetically characterize *Trypanosoma cruzi* in a black bear (*Ursus americanus*) cub.

Gene target	Primers (5′-3′)	Cycle parameters	Reference
24Sα rRNA	D71: AAGGTGCGTCGACAGTGTGG D72: TTTTCAGAATGGCCGAACAGT	30× 94C 1 min 60C 1 min 72 1 min	Souto et al., 1996
dihydrofolate reductase-thymidylate synthase (DHFR-TS)	DH1S: CGCTGTTTAAGATCCGNATGCC DH3A: CGCATAGTCAATGACCTCCATGTC	29× 94 30 s 58 1 min 72 2 min	Machado and Ayala, 2001
cytochrome oxidase subunit II and NADH dehydrogenase subunit I (COII-ND1)	ND1.3A: GCTACTARTTCACTTTCACATTC COII.2A: GCATAAATCCATGTAAGACMCCACA	45 x 94C 45 s 58 1 min 94 2 min	Machado and Ayala, 2001

International (San Antonio, Texas) that is designed to detect antibodies in human samples. Serum samples from the *T. cruzi* black bear cub case were run with the serologic assays and after being shown to be positive, were subsequently used as a positive control for these three assays.

3. Results

Black bears with *T. cruzi* exposure were detected in all three states. In total, 41 of the 198 samples tested (20.7%) were positive for antibodies to *T. cruzi* with at least one of the serologic assays (Fig. 3, Table 2). Assuming a bear was positive if positive on a single assay, based on Fischer's exact test, there was no difference in prevalence between the three states ($p > 0.05$); however, prevalence was highest in Pennsylvania and lowest in North Carolina (Fig. 3). For Pennsylvania, 12 of 44 (27.3%) samples were positive on at least one assay, with three being positive by ELISA (6.8%), two positive by ICT (4.5%), and eight positive by IFA (18.2%). Of the 8 IFA-positive samples, one was positive at a 1:80 dilution, one was positive at 1:1280, and 6 were positive at a 1:20 dilution. A single bear was positive with both the ELISA and IFA tests; the IFA titer of this sample was 1:1280. For North Carolina, six of the 44 samples (13.6%) were positive by either ELISA, IFA, or both. Of the 44 serum samples tested, one was positive by ELISA (2.3%) and six were positive by IFA (13.6%), all being positive at a 1:20 dilution. One bear was positive by both assays and all samples were negative by ICT. For California, 23 of 110 (20.9%) bears were positive with at least one assay. Because of the presumed difference in diversity and density of vectors in southern and northern California (Bern et al., 2011; Curtis-Robles et al., 2018b), we also analyzed the data by region. In northern California, 12 out of 59 (20.8%) samples were positive by either ELISA or IFA with 11 being positive by ELISA (18.6%) and one positive by IFA at a 1:20 dilution (1.7%). In Southern California, 11 of 51 samples (21.6%) were positive on at least one assay with nine being positive by ELISA (17.6%), one (2.0%) by IFA at a 1:40 dilution, and 4 by ICT (7.8%). Two of the samples positive by ICT were positive by ELISA, and one was positive by IFA as well. The individual testing results for the 41 bears positive by at least one assay are shown in Supplemental Table 1.

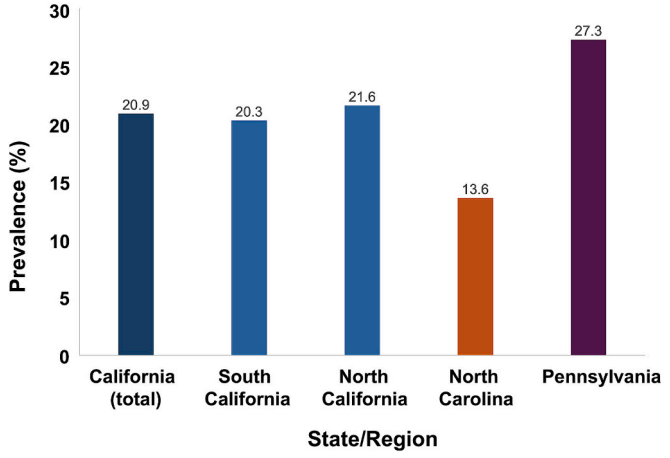

Fig. 3. Summary of *Trypanosoma cruzi* prevalence rates by location. Positive samples are defined as those positive by at least one assay. Note the maximum value of the y-axis is 30%.

4. Discussion

This study describes a case of *T. cruzi* infection in a young black bear from California. The cub presented with clinical signs (lethargy and poor weight gain) that could be attributed to acute *T. cruzi* infection, but it could have been due to the proliferative bony lesions that were later noted. However, the bear was anemic and had elevated GGT liver enzymes, both of which have been reported during acute Chagas disease (Schielke et al., 2002; Durães-Oliveira et al., 2024). Blood smear analysis and PCR testing of whole blood showed that parasitemia rapidly decreased after initial diagnosis. Also, the bear cub was positive for antibodies to all three assays used in this study and remained

seropositive after ~9 mo which is consistent with other studies showing that antibodies are persistent for extended periods following infection (Lidani et al., 2017). The only other reports in ursids were a fatal acute case in a captive polar bear (*Ursus maritimus*) in Mexico and a captive Andean bear (*Tremarctos ornatus*) in Brazil (Jaime-Andrade et al., 1997; Reis et al., 2020).

Following the detection of this clinical case, a serologic survey was conducted to determine the prevalence of *T. cruzi* exposure of black bears. Because no serologic assay has been validated for use in bears, three different assays were used. The prevalence of *T. cruzi* antibodies was moderately high (~21%) if a bear was considered positive if only one assay was positive. However, the prevalence was much lower (1%), if a bear was only considered positive if two or three of the assays were positive. Because no tests have been validated, the actual seroprevalence is unknown, but these data indicate that black bears are naturally exposed to *T. cruzi* across the United States (i.e., exposed bears were detected in all three states). Interestingly, one of the *T. cruzi* positive samples from northern California was collected in 1978 highlighting the importance of archived samples in pathogen surveillance studies and indicates that *T. cruzi* exposure of bears has been occurring for a long period of time. The potential range of prevalence (1% to 21%) is similar to studies on several other wildlife species such as raccoons, striped skunks, Virginia opossums, coyotes (*Canis latrans*), and foxes (Karsten et al., 1992; Pung et al., 1995; Hancock et al., 2005; Brown et al., 2010; Charles et al., 2013; Curtis-Robles et al., 2016).

Based on PCR testing and sequence analysis of multiple genetic targets, the bear cub was infected with *T. cruzi* type IV lineage. The TcIV DTU detected in the bear cub and other US wildlife and domestic dogs are distinct from South American strains, suggesting it independently evolved in North America, and it is often referred to as TcIV-USA (Roellig et al., 2013; Flores-López et al., 2022). The TcIV DTU has a strong association with the order Carnivora and is the main lineage responsible for infections of domestic dogs (Roellig et al., 2008; Patel et al., 2012; Roellig et al., 2013; Brenière et al., 2016; Izeta-Alberdi et al., 2016; Curtis-Robles et al., 2018a; Hodo et al., 2020; Meyers et al., 2020; McCain et al., 2023). The Type I lineage is more often detected in opossums, domestic cats (*Felis catus*), and is responsible for human infections in North America, although the South American TcIV DTU can also infect people (Dorn et al., 2007; Roellig et al., 2009; Roellig et al., 2010; Monteiro et al., 2012; Roellig et al., 2013; Brenière et al., 2016; Zecca et al., 2020; Dumonteil et al., 2021; Rodriguez et al., 2021; Freitas et al., 2023). Co-infections are frequently detected in vectors, non-human primates, domestic dogs, some rodent species (e.g., *Neotoma* spp.), and striped skunks (Charles et al., 2013; Herrera et al., 2015; Curtis-Robles et al., 2017; Curtis-Robles et al., 2018b; Hodo et al., 2018). Other DTUs have occasionally been detected in the southern US (Dumonteil et al., 2020). Because we only had a single bear tested by PCR, it is unknown if bears can also become infected with the TcI DTU; additional molecular and/or culture-based surveillance would be needed to evaluate if both DTUs can infect bears, similar to data from non-human primates, dogs, woodrats and skunks (Roellig et al., 2010; Charles et al., 2013; Padilla et al., 2021).

There was a high degree of discordant results between the three serologic assays used in this study, but discordant results are common among people and animals with *T. cruzi* infections (Castro Eiro et al., 2017; Meyers et al., 2017). The IFA and ICT tests have had high agreement when used to test validated species, such as domestic dogs (Nieto et al., 2009). Historically, ELISA testing had high agreement with other tests along with high sensitivity and specificity for testing humans (Malan et al., 2006). With regards to wildlife, it is typically described as the best assay for large-scale testing, but the lack of species-specific commercial kits is widely acknowledged as a challenge for diagnostic work in wildlife (Dorion et al., 2021). The ELISA kit we used was validated for human use while the IFA and ICT were designed for use on domestic dogs. The variable cross-reactivity between black bear IgG and the anti-human and anti-dog antibodies used in the ELISA and IFA assays, respectively, could explain discordant results. Another factor potentially associated with discordant results is the degree of hemolysis noted in some samples and repeated freeze-thaw cycles, both of which can impact antibody detection (Boadella and Gortázar, 2011). Regardless, two bear samples were positive on all three assays including the clinical case cub and an additional bear from southern California.

5. Conclusions

In conclusion, this study confirms that American black bears are susceptible to infection with *T. cruzi* and are naturally exposed across multiple states. The initial case had clinical signs suggestive of acute Chagas disease, but the bear recovered with a concurrent decrease in parasitemia. Molecular characterization confirmed the bear cub was infected with TcIV DTU, which infects numerous species of wildlife, domestic dogs and non-human primates. The use of several serological assays validated for use in humans and domestic dogs suggest that bears are exposed to *T. cruzi* in the wild and prevalence is likely low, but discordant results were common. Future studies using PCR testing of tissues or whole blood would be needed to better understand the prevalence *of T. cruzi* in wild black bears and what lineages are most commonly associated with infection.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2024.101129.

Ethical statement

No animals were specifically harmed for the purpose of this study but were used opportunistically and abided by institutional IACUCs.

CRediT authorship contribution statement

Reece Hughes: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Raquel Francisco:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis. **Kayla Garrett:** Writing – review & editing, Investigation. **Kevin Willitts:** Writing – review & editing, Investigation, Funding acquisition. **Brandon Munk:** Writing – review & editing, Investigation, Conceptualization. **Carlos Rodriguez:** Writing – review & editing, Investigation. **Alexa Rosypal von Dohlen:** Writing – review & editing, Investigation. **Sterling McCarrall:** Writing – review & editing, Investigation. **T'Keyah Dennard:** Writing – review & editing, Investigation. **Timothy Champion:** Writing – review & editing, Investigation. **Tracy Brown-Fox:** Writing – review & editing, Investigation. **Jennifer Strules:** Writing – review & editing, Investigation. **Colleen Olfenbuttel:** Writing – review & editing, Investigation. **Christopher DePerno:** Writing – review & editing, Investigation. **Sarah A. Hamer:** Writing – review & editing, Methodology, Investigation. **Michael J. Yabsley:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

We thank the biologists who collected the serum samples used in this study and the staff at Lake Tahoe Wildlife Care for providing excellent care to the bear cub. This work was partially funded by SCWDS member state wildlife agencies through the Federal Aid to Wildlife Restoration Act (50 Stat. 917). Further support was provided by SCWDS federal wildlife agency partners, including the United States Fish and Wildlife

Service National Wildlife Refuge System, the United States Geological Survey Ecosystems Mission Area, and National Science Foundation: Historically Black Colleges and Universities Undergraduate Program (HBCU-UP) No. 1818708. For North Carolina, this project was funded by the Pittman-Robertson Federal Aid to Wildlife Restoration Grant and was a joint research project between the North Carolina Wildlife Resources Commission and the Fisheries, Wildlife, and Conservation Biology Program at North Carolina State University.

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