

*Case Report*

**ENTAMOEBA SP. INFECTION IN A BEARDED DRAGON  
(*POGONA VITTICEPS*)**

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**Abstract**

A 3-year-old, male intact, pet inland bearded dragon (*Pogona vitticeps*) presented with a history of diarrhea, progressive inappetence and weight loss. A palpable cranial celomic mass was identified on physical examination and confirmed to be hepatic in origin by celomic ultrasonography. Hematologic and biochemical abnormalities were mild and consistent with inflammation, regenerative anemia, and hepatocellular injury. Fine needle aspiration of the liver masses was suggestive of amoebiasis and the patient was humanely euthanized. PCR and Sanger DNA sequencing of liver aspirates were supportive of *Entamoeba* infection, although definitive speciation was not possible. Pathogenic amoebiasis due to infection by *E. invadens* has been reported in a wide range of reptiles and is an important cause of morbidity and mortality in these species.

**Key words:** *Entamoeba*, enteritis, liver abscess, *Pogona vitticeps*, reptilia

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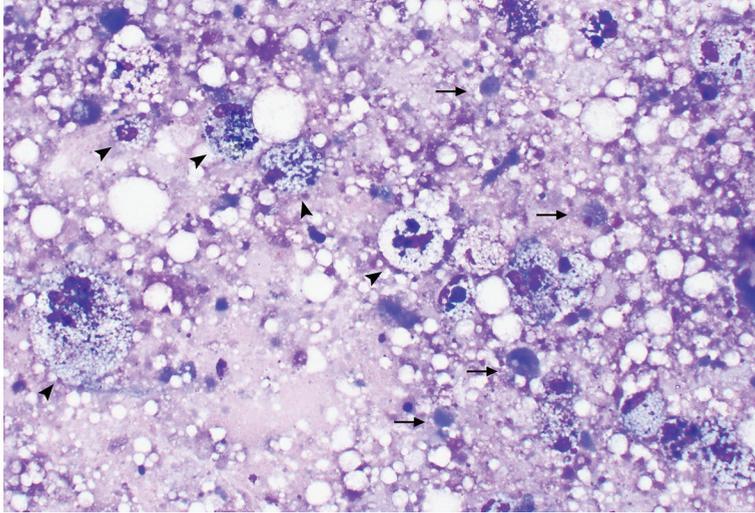
## CASE PRESENTATION

A 3-year-old, male intact, pet inland bearded dragon (*Pogona vitticeps*) presented to the Exotics and Zoological Animal Medicine Service at Kansas State University following several months of diarrhea, decreased appetite progressing to anorexia, and weight loss. On physical examination, the patient was quiet and alert with a palpable mass in the cranial celom. Complete blood count revealed a mild heterophilia (7,800/uL) with mild toxic change, mild monocytosis (2,100/uL) and a mild lymphopenia (1,800/uL), consistent with inflammation. There was also a mild anemia (PCV 18%) with mildly increased numbers of immature erythrocytes. Serum chemistry revealed a mild increase in AST (44 U/L) (Ellman 1997, Tamukai et al. 2011). Two view whole body radiographs were performed and a large soft tissue opacity mass (41 x 20 mm) was observed filling the cranial celomic cavity with resultant dorsal displacement and compression of the trachea. Another large, mid-celomic mass was observed (55 x 30 mm). There was a diffuse interstitial to coalescing nodular pulmonary pattern and body condition was assessed to be thin given the absence of caudal fat bodies. Celomic ultrasonography confirmed an irregularly marginated echogenic mass caudal to the heart and multiple well-circumscribed hypo to mixed echogenic mass lesions within the liver (Figure 1). A moderate amount of echogenic effusion was also observed within the celomic cavity and within the pericardial sac. Fine needle aspiration of the hepatic masses was performed. The sample was of low intact nucleated cellularity and consisted mostly of necrotic and apoptotic cell debris with frequent vacuolated



**Figure 1.** Sagittal ultrasonographic image of a pet inland bearded dragon (*Pogona vitticeps*) demonstrating a mass associated with the liver. The white arrows outline the borders of the mass. CR indicates the cranial aspect of the coelom, CD indicates the caudal aspect of the coelom, and V indicates the ventral aspect of the coelom.

and debris laden macrophages (Figure 2), few intact hepatocytes, and rare structures that were concerning for, but not diagnostic of, amoeba trophozoites. Based on these findings *Entamoeba invadens* infection was suspected and the owners elected humane euthanasia without necropsy.



**Figure 2.** Wright-Giemsa stained liver aspirate from a pet inland bearded dragon (*Pogona vitticeps*). Highly vacuolated macrophages (black arrowheads) are observed within a purple coarsely stippled background with acellular lipid droplets and necrotic cellular debris (black arrows). 50x.

The air-dried Wright-Giemsa stained liver aspirates were submitted to the Free-Living and Intestinal Amebas (FLIA) Laboratory within the Waterborne Disease Prevention Branch of the U.S. Centers for Disease Control and Prevention (CDC). The sample was negative for *E. histolytica* and *E. dispar* by the diagnostic duplex real-time polymerase chain reaction (PCR) assay (Qvarnstrom et al., 2005). Generic *Entamoeba* sp. conventional PCR assay (Stensvold et al., 2011) was positive, and Sanger sequencing results (3'-ended 57-bp clean sequence: CCCTTCCGTCAAATTTCTTTAAGTTCAG CCTTGTGACCATACTCCCCCTGAAGTAAG, GenBank accession number MN 879388) were 100% homologous with *Entamoeba muris*; however, repeated amplification and sequencing attempts to confirm these findings were unsuccessful.

## DISCUSSION

*Entamoeba muris* is a non-pathogenic species of amoeba that colonizes the intestine of wild and domestic rodents (Hooshyar, Rostamkhani, and Rezaeian, 2015). *E. muris* has been identified in feces of captive tortoises (Wolf et al., 2014), and in this context is assumed to transit through the intestinal tract of these individuals, presumably following access in the environment to rodent feces containing cysts. Identification of *E. muris* DNA within a liver aspirate in the case of this pet inland bearded dragon

(*Pogona vitticeps*) would suggest an invasive biological behavior, although these results could not be confirmed, possibly due to poor DNA quality, as the assay was not developed for use with fine needle aspirate samples. It is also possible that the *Entamoeba* species identified in this sample shares some homology with *E. muris* but is in fact a distinct species and a pathogen. The clinical presentation and imaging findings in this case were suggestive of *E. invadens* (Gardhouse et al., 2015), an established pathogen in reptiles, and thus, further discussion will focus on this species.

*E. invadens* is an amoebozoan organism, which can act as either a gastrointestinal commensal or a pathogen, in many species of reptiles. *E. invadens* has a direct life cycle in which cysts are ingested directly from feces or from a contaminated environment. Excystation occurs within the host intestine releasing the motile trophozoites, which replicate and can either remain within the intestinal lumen feeding on ingesta, or can invade the mucosa resulting in ulcerative enterocolitis and diarrhea. Invasion of adjacent lymphatic and blood vessels can result in embolization of trophozoites to the liver via the portal vein, followed by widespread dissemination to distant sites including lung, spleen, kidney, and brain (Bonner, 2001; Kojimoto et al., 2001; Gardhouse et al., 2015; Park et al., 2019). Secondary localized or disseminated bacterial infections (typically involving enteric organisms) can be present and contribute to morbidity in affected individuals. Factors that influence encystation have not been entirely elucidated, although ambient temperature has been shown to affect cyst formation in experimentally infected Eastern garter snakes (*Thamnophis sirtalis*) (Meerovitch, 1961). Trophozoites rapidly degrade outside the body (Brewer et al. 2008), but cysts can remain viable and infective in the environment for weeks to months (Bradford et al., 2008). While environmental decontamination protocols have not been established, *E. histolytica* cysts are rapidly killed when exposed to high temperatures (52°C/126°F), so steam and hot water could be effective against *E. invadens* cysts (Bonner, 2001).

Aquatic reptiles, especially chelonians and crocodylians (Bonner, 2001; Brewer et al., 2008; Garcia et al. 2014), and herbivorous species (Kojimoto et al., 2001) are typically subclinical carriers, although young, old, immunocompromised, or otherwise debilitated individuals (such as following capture and/or transport) of any reptilian species can develop clinical disease. Snakes and lizards appear to be especially prone to clinical illness (Bonner, 2001). *E. invadens* is highly contagious and has caused outbreaks in juvenile (MacNeill et al., 2002) and wild-caught (Ozaki et al., 2000) chelonians, and in captive pythons (Kojimoto et al., 2001). Pathogenicity is also dependent on differences in strain virulence and ambient host temperature (Meerovitch, 1961; Garcia et al., 2014). The most common presenting complaints in affected reptiles are decreased appetite progressing to anorexia, regurgitation, and diarrhea, although some individuals present dead or acutely moribund (Ozaki et al., 2000; Bonner, 2001; Kojimoto et al., 2001; MacNeill et al., 2002; Brewer et al., 2008; Baseler et al., 2014). Hematologic and biochemical abnormalities associated with *E. invadens* are poorly characterized. One report in a green iguana documented similar hematologic findings as we observed in our case including decreased PCV with evidence of regeneration

and a moderate leukocytosis due to heterophilia and monocytosis, with toxic change and a left shift (Nikousefat, 2014).

Fresh fecal smears can be used for *ante mortem* diagnosis of *E. invadens* (Bonner, 2001; Garcia et al., 2014); however, false negatives are possible as animals can shed intermittently or have low burden infections. Multiple fecal examinations could be needed to identify trophozoites and/or cysts; however, in general, fecal examination is a relatively insensitive test for *E. invadens*. Iodine wet mounts of fresh feces (Garcia et al., 2014) or fixation of fecal smears with polyvinyl alcohol followed by trichrome and iron hematoxylin staining has been reported to highlight the organisms (Bonner, 2001); however, other non-pathogenic amoeba can have a similar appearance (Bradford et al., 2008; Garcia et al., 2014; Park et al., 2019). Trophozoites can be cultured from fresh feces using Robinson's medium, and this is a more sensitive method than direct fecal examination, but culture is time consuming and Robinson's medium can selectively exclude the growth of concurrent pathogens (Garcia et al., 2014). PCR is a sensitive technique for identifying *E. invadens* in feces (Bradford et al., 2008) and tissues (Chia et al., 2009; Park et al., 2019), although might not be sufficiently specific to exclude other fecal protozoa, such as *Blastocystis* species (Garcia et al., 2014). DNA sequencing is, therefore, recommended to identify species in positive PCR samples. Unfortunately, there are no commercially available PCR assays for *E. invadens*. Serologic methods of detecting *E. invadens* have yet to be developed and positive fecal samples from reptiles did not cross-react with an *E. histolytica* ELISA (Brewer et al., 2008).

For animals that succumb to infection or are euthanized, the most commonly reported gross findings on necropsy are colonic edema and multifocal to coalescing gastrointestinal ulcers, sometimes with concurrent mucosal pseudomembrane formation (Meerovitch, 1961; Ozaki et al., 2000; Bonner, 2001; Kojimoto et al., 2001; Nikousefat, 2014; Park et al., 2019). Generalized hepatomegaly is also frequently reported (Ozaki et al., 2000; MacNeill et al., 2002; Chia et al., 2009; Basler et al., 2014; Gardhouse et al., 2015; Park et al., 2019). Trophozoites can be readily identified in histologic sections of affected tissues, and are highlighted by PAS (Park et al., 2019) and silver stains (Kojimoto et al. 2001; Baseler et al., 2014). PAS and trichrome staining has also been described as highlighting trophozoites in emulsified and polyvinyl alcohol-fixed liver samples (MacNeill et al., 2002). Other histologic findings include necrosis and hemorrhage with mixed inflammation including heterophils, lymphocytes, macrophages, and eosinophils (Ozaki et al., 2000; Kojimoto et al., 2001; Baseler et al., 2014). Fluorescent antibodies against *E. invadens* applied to formalin-fixed tissues can be helpful in confirming infection, although background staining can render interpretation challenging (Baseler et al., 2014; Kojimoto et al., 2001).

*E. invadens* is an important contributor to morbidity and mortality in reptiles. While a variety of *ante mortem* diagnostic tests are available, most are insufficiently specific to distinguish *E. invadens* from other non-pathogenic amoeba, and thus, PCR followed by DNA sequencing remains the most reliable *ante mortem* method for confirming infection. Unfortunately, there is no commercially available PCR assay for *E. invadens*.

Fine needle aspirates of affected tissues can aid in the *ante mortem* diagnosis of amoebic infections in reptiles, even if trophozoites cannot be confirmed morphologically, by providing material for PCR and DNA sequencing. Identification of trophozoites within histologic sections (collected via biopsy or at the time of necropsy) of intestine, liver, and other tissues also confirms pathogenic amoeba infection, and trophozoites can be highlighted by special stains. Unfortunately, once clinical signs have developed, treatment is often ineffective (Ozaki et al., 2000; MacNeill et al., 2002; Garcia et al., 2014). Care to avoid potential exposure by limiting mixed-housing of different reptilian species, especially of chelonians with snakes and lizards, is recommended.

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### Authors contributions

All authors contributed to manuscript preparation and approval. DS additionally contributed the photomicrographs of the liver aspirate cytology.

### Competing interests

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of CDC.

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## ENTAMOEBA SP. INFEKCIJA KOD BRADATE AGAME (*POGONA VITTICEPS*)

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### Kratak sadržaj

Trogodišnji nekastrirani mužjak bradate agame (*Pogona vitticeps*) koji se drži kao kućni ljubimac je doveden na kliniku zbog dugotrajne dijareje, progresivnog gubitka apetita i težine. Kliničkim pregledom je otkrivena palpabilna kranijalna celomska masa i ultrazvukom je utvrđeno da čini deo jetre. Poremećaji hematoloških i biohemijskih parametara su bili blagi i ukazivali su na inflamaciju, regenerativnu anemiju i hepatocelularna oštećenja. Tankoiglenom biopsijom mase otkrivene na jetri ukazivala je na amebijazu i pacijent je eutaniziran. PCR i DNK sekvencioniranje su takođe ukazivali na infekciju Entamoeba-om, mada definitivno određivanje vrste nije bilo moguće. Patogena amebijaza usled infekcije sa *E. invadens* je dokazana kod velikog broja vrsta gmizavaca i značajan je uzročnik uginuća kod ovih vrsta životinja.

**Ključne reči:** Entamoeba, enteritis, apsces jetre, *Pogona vitticeps*, reptilia