



Short Communication

Morphological and molecular characterization of an uninucleated cyst-producing *Entamoeba* spp. in captured Rangeland goats in Western Australia



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ABSTRACT

Uninucleated *Entamoeba* cysts measuring $7.3 \times 7.7 \mu\text{m}$ were detected in faecal samples collected from wild Rangeland goats (*Capra hircus*) after arrival at a commercial goat depot near Geraldton, Western Australia at a prevalence of 6.4% (8/125). Sequences were obtained at the 18S rRNA (n = 8) and actin (n = 5) loci following PCR amplification. At the 18S locus, phylogenetic analysis grouped the isolates closest with an *E. bovis* isolate (FN666250) from a sheep from Sweden with 99% similarity. At the actin locus, no *E. bovis* sequences were available, and the isolates shared 94.0% genetic similarity with *E. suis* from a pig in Western Japan. This is the first report to describe the morphology and molecular characterisation of *Entamoeba* from Rangeland goats in Western Australia and the first study to produce actin sequences from *E. bovis*-like *Entamoeba* sp.

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1. Introduction

Organisms of the genus *Entamoeba* have adapted to live as parasites or commensals in the digestive tract of humans and other mammals, birds, amphibians, fish and reptiles (Skirnisson and Hansson, 2006; Stensvold et al., 2010). Species within the genus can all be assigned to either uni-, quadri- or octo-nucleated and non-cyst-producing morphological groups (Stensvold et al., 2010): 1) species without cysts (*E. gingivalis*-like group), 2) species with uninucleated cysts (*E. bovis*-like group), 3) species with quadrinucleated cysts (*E. histolytica*-like group, 4) octonucleated cysts (*E. coli*-like group). Several species are found in humans and animals with the quadrinucleate *E. histolytica* responsible for invasive ‘amoebiasis’ (which includes amoebic dysentery and amoebic liver abscesses) in humans.

Uninucleated cyst-producing Entamoebae have been isolated from a range of hosts including humans, non-human primates, other mammals and birds (Noble and Noble, 1952; Skirnisson and Hansson, 2006; Stensvold et al., 2010). Ruminants such as cattle and sheep are common hosts of uninucleate cyst-producing Entamoebae (Noble and Noble, 1952; Jacob et al., 1990; Hampton et al., 2006; Skirnisson and Hansson, 2006; Kanyari et al., 2009;

Stensvold et al., 2010; Stensvold et al., 2011) and unidentified *Entamoeba* species have been reported in goats in Kenya (Kanyari et al., 2009), Thailand (Sangvaranond et al., 2010), Tanzania (Mhoma et al., 2011), Cameroon (Ntonifor et al., 2013) and Brazil (Radavelli et al., 2014).

Until recently, the detection, identification and assignment of *Entamoeba* organisms to species relied mainly on morphology and the host in which parasites were identified (Stensvold et al., 2010; Stensvold et al., 2011). However, morphology is not a reliable tool for delimiting *Entamoeba* species as cyst morphology varies substantially within as well as between uninucleated cyst-producing species from different ruminant hosts (Noble and Noble, 1952; Pillai and Kain, 1999; Stensvold et al., 2010). The use of molecular tools is therefore essential to resolve the identification, taxonomy, epidemiology and clinical significance of *Entamoeba* species without reliance on parasite cultures or experimental infections (Stensvold et al., 2011; Jacob et al., 2016).

Rangeland goats are an introduced animal species in Australia. They can be legally trapped and reared by licensed operators (goat depots) for the domestic and export meat markets, which was worth approximately \$AUS242 million in 2014 (MLA, 2015). Few studies have conducted genetic characterisation of *Entamoeba* species from ruminants (Stensvold et al., 2010; Jacob et al., 2016), which is important for understanding their evolutionary and taxonomic relationships. In the present study, uninucleate *Entamoeba* cysts were identified in the faeces of Rangeland goats in Western

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Table 1Morphometric characteristics of uninucleated cyst-producing *Entamoeba* spp. reported from livestock compared with the *Entamoeba* cysts isolated from Rangeland goats in Western Australia in the present study.

Species ^a	Host	Cyst diagnostic characters						Reference
		Cyst shape	Cyst size (mean)	Nucleus (mean)	Karyosome	Glycogen	Vacuoles	
<i>Entamoeba bovis</i>	Cattle (<i>Bos taurus</i>)	N/A	4–15 µm (8.8 µm)	1.5–5.5 µm (3.0 µm)	N/A	N/A	present in various sizes	Noble and Noble (1952) Kingston and Stabler (1978)
	White-tailed deer (<i>Odocoileus virginianus</i>)	N/A	6–11 µm (8.2 µm)	N/A	N/A	N/A	N/A	
	Gnu (<i>Connochaetes taurinus</i>)	N/A	6–13 µm (9.0 µm)	N/A	N/A	N/A	N/A	Mackinnon and Dibb (1938) Bray (1964)
	Bay Duiker (<i>Cephalophus dorsalis</i>)	N/A	N/A	N/A	N/A	N/A	N/A	
	Cattle (<i>Bos taurus</i>)	N/A	3.9–14.4 µm (6.6 µm)	N/A	N/A	N/A	N/A	Stensvold et al. (2010) Stensvold et al. (2010)
	Sheep (<i>Ovis aries</i>)	N/A	5.4–13.8 µm (7.2 µm)	N/A	N/A	N/A	N/A	
<i>Entamoeba ovis</i> ^b	Sheep (<i>Ovis aries</i>)	N/A	4–13 µm (7.2 µm)	N/A	N/A	N/A	N/A	Noble and Noble (1952)
<i>Entamoeba deblickei</i>	Goat (<i>Capra hircus</i>)	round/oval	4–12 µm (6.42 µm)	N/A (2.4 µm)	N/A	present	present	Nieschulz (1923) Noble and Noble (1952) Hoare (1940)
	Goat (<i>Capra hircus</i>)	spherical/ovoid/ellipsoid	4.75–13.3 6.65 µm ^c	1.9–4.2 µm N/A	large central/eccentric (off to the side)	N/A	present	
<i>Entamoeba dilimani</i> ^b	Goat (<i>Capra hircus</i>)	N/A	5–16 µm (9.7 µm)	N/A	N/A	N/A	N/A	Noble (1954)
<i>Entamoeba suis</i> ^b	Pig (<i>Sus domesticus</i>)	N/A	9.5–15.5 µm (12.85 µm)	N/A	N/A	N/A	N/A	Clark et al. (2006)
<i>Entamoeba polecki</i> ^b	Pig (<i>Sus domesticus</i>)	N/A	4–17 µm (8.09 µm)	large (2.22 µm)	N/A	N/A	N/A	Noble and Noble (1952)
<i>Entamoeba</i> from rangeland goats	Goat (<i>Capra hircus</i>)	spherical	6.5–12.3 µm (7.3 × 7.7 µm)	(1.3–2.9) 1.7 µm	central	diffuse	present	Present study

^a The non-cyst species *E. caprae*, has been reported in a goat (Fantham, 1923), however as only trophozoite and its nucleus measurements were given, this species was not included.

^b Based on morphological data, *Entamoeba ovis*, *E. suis*, *E. polecki* and *E. dilimani* were previously considered synonymous with *E. deblickei* (Levine, 1985). However, molecular data has shown that *E. polecki* and *E. suis* are not synonymous (Clark et al., 2006).

^c This is the median of 800 cysts measured in English goats and believed to be *E. deblickei* (Hoare, 1940).

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