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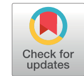
Short communication

# A newly described strain of *Eimeria arloingi* (strain A) belongs to the phylogenetic group of ruminant-infecting pathogenic species, which replicate in host endothelial cells *in vivo*



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ABSTRACT

Coccidiosis caused by *Eimeria* species is an important disease worldwide, particularly in ruminants and poultry. *Eimeria* infection can result in significant economic losses due to costs associated with treatment and slower growth rates, or even with mortality of heavily infected individuals. In goat production, a growing industry due to increasing demand for caprine products worldwide, coccidiosis is caused by several *Eimeria* species with *E. arloingi* and *E. ninakohlyakimovae* the most pathogenic. The aims of this study were genetic characterization of a newly isolated European *E. arloingi* strain (A) and determination of phylogenetic relationships with *Eimeria* species from other ruminants. Therefore, a DNA sequence of *E. arloingi* strain (A) containing 2290 consensus nucleotides (the majority of 18S rDNA, complete ITS-1 and 5.8S sequences, and the partial ITS-2) was amplified and phylogenetic relationship determined with the most similar sequences available on GenBank. The phylogenetic tree presented a branch constituted by bovine *Eimeria* species plus *E. arloingi*, and another one exclusively populated by ovine *Eimeria* species. Moreover, *E. arloingi*, *E. bovis* and *E. zuernii*, which all replicate in host intestinal endothelial cells of the lacteals, were found within the same cluster. This study gives new insights into the evolutionary phylogenetic relationships of this newly described caprine *Eimeria* strain and confirmed its close relationship to other highly pathogenic ruminant *Eimeria* species characterized by macromeront formation in host endothelial cells of the central lymph capillaries of the small intestine.

## 1. Introduction

Currently, more than 1200 *Eimeria* species are known (Chapman et al., 2013) and it is assumed that many more remain to be discovered (Blake, 2015). The great majority of these species are monoxenous enteropathogens of vertebrates which usually induce only mild pathology and mild or non-clinical disease. Nonetheless, certain species such as *E. bovis*, *E. zuernii*, *E. alabamensis* (cattle), *E. ovinoidalis*, *E. bakuensis* (sheep), *E. cameli, E. dromedari* (camels), *E. ninakohlyakimovae* and *E. arloingi* (goat) are considered highly pathogenic, defined by the formation of macromeronts, and severe intestinal lesions.

Worldwide, coccidiosis is particularly relevant to ruminant and poultry production (Chapman et al., 2013; Daugschies and Najdrowski, 2005). The economic impact in both industries is enormous and was recently valued as a 6–9% reduction in gross margin for ruminants, and to exceed US$3 billion for poultry (Blake and Tomley, 2014; Lassen and Ostergaard, 2012). Costs of prevention and treatment, combined with the morbidity and mortality of heavily infected individuals, are the main factors influencing economic losses. Every year, more than one billion goats are reared worldwide (FAOSTAT, 2014) and coccidiosis constitutes a major concern for the caprine industry.

Historically, morphology of sporulated oocysts has been largely used for identification of distinct *Eimeria* species (Levine, 1985), but recently molecular characterization has been widely used to clarify precise species classification, particularly where morphological differentiation is difficult due to similarities in shape and size (Kokuzawa et al., 2013; Ogedengbe et al., 2011). Therefore, the aim of this study was to analyse a newly described European *E. arloingi* strain (A) isolated

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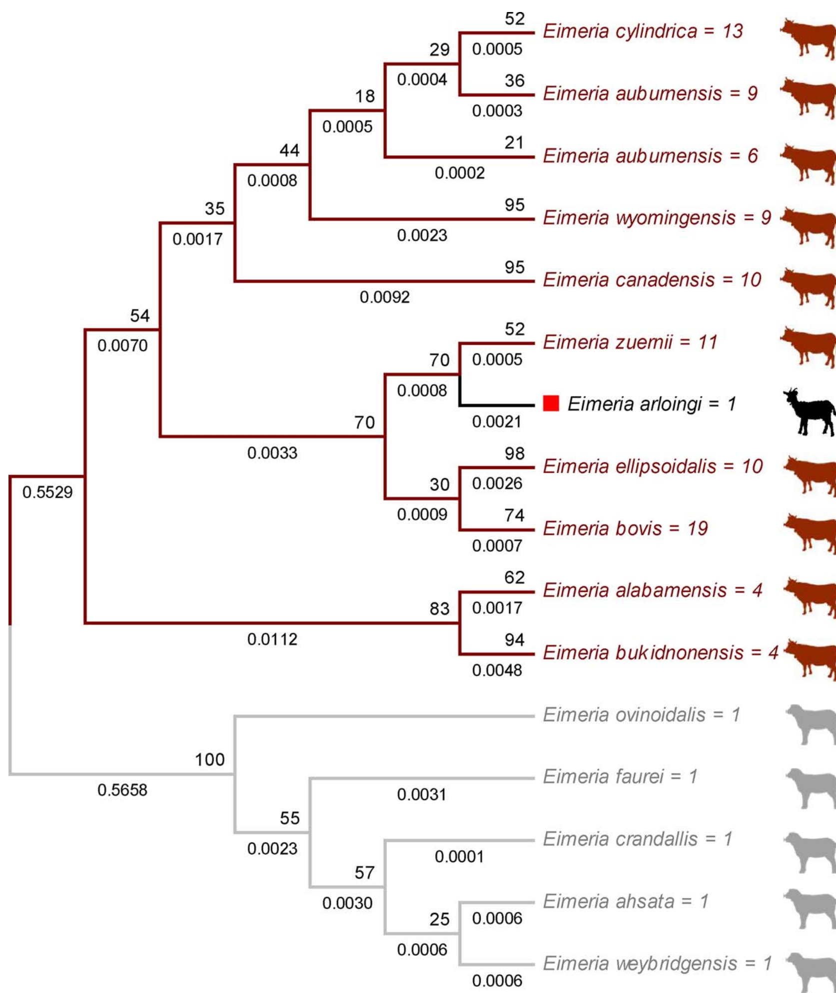


**Fig. 1.** Neighbor-Joining phylogenetic tree generated using an *E. arloingi* partial 18S-ITS1-5.8S- partial ITS2 sequence and the 100 most similar sequences available in GenBank as of 9th May 2016 (see supplementary data). The consensus of 1000 bootstrap replicates is shown. The sequence of interest *E. arloingi* is marked with a red square. The number at the end of each node indicates how many sequences constitute each of the collapsed branches. The host species of each parasite is shown: bovine (cow drawing); ovine (sheep drawing); caprine (goat drawing). Maximum Likelihood and Minimum Evolution methods achieved comparable topologies. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from Portugal (Silva et al., 2015) and investigate its phylogenetic relationship to other *Eimeria* species which infect ruminants.

## 2. Material and methods

### 2.1. Parasites

*E. arloingi* (strain A) oocysts were isolated from naturally infected goat kids and passaged in male White German goat kids as previously described (Silva et al., 2015). Isolated oocysts were allowed to sporulate at RT in a 2% (*w/v*) potassium dichromate solution (Hermosilla et al., 2002) and then stored at 4 °C until further use.

### 2.2. Purification of oocysts

Sporulated *E. arloingi* oocysts were washed to remove all traces of potassium dichromate. Three million oocysts were pelleted (750 × *g*, 10 min) and re-suspended in 5% sodium hypochlorite, swirling intermittently. After 10 min of treatment oocysts were washed with tap water (750 × *g*, 10 min). The supernatant was removed and the pellet was re-suspended in saturated salt. The 100 ml vessel was filled up to 2 cm from the top, overlaid with Milli-Q water and centrifuged as before. Oocysts present at the interface between the salt and water phases were collected and washed three times. After the final wash, the *E. arloingi* purified oocysts were re-suspended in Milli-Q water and stored at 4 °C.

### 2.3. DNA extraction

Approximately three million purified sporulated *E. arlongi* oocysts were chilled on ice and homogenized using a Mini Beadbeater-8 (Biospec Products, Bartlesville, USA) with an equal volume of sterile glass beads (0.4–0.6 mm, Sigma, Gillingham, UK), at 3000 oscillations/min. Subsequently, genomic DNA was extracted with TRIzol® Reagent (VWR, Carlsbad, USA) according to the manufactureŕs instructions, re-suspended in 20 µl MQ water and stored at −20 °C until further use.

### 2.4. Polymerase chain reaction (PCR), molecular cloning and sequencing

PCR amplification was performed using Taq DNA Polymerase (Invitrogen, California, USA), as previously described (Marugan-Hernandez et al., 2016), with the primers ERIB1, ERIB10, EITSF2 and EITSR2 [sequences as described elsewhere (Honma et al., 2011; Schwarz et al., 2009); synthesized by Sigma-Aldrich, Gillingham, UK]. PCR products were evaluated by agarose gel electrophoresis and cloned into pGEM®-T Easy (Promega, Southampton, UK). Plasmids were propagated in *Escherichia coli* Fast-Media® (InvivoGen) and colonies were picked in triplicate for purification using a QIAprep® Spin Miniprep Kit (Qiagen, Hilden, Germany) and sequenced (GATC Biotech, Konstanz, Germany) as described by the respective manufacturers. The consensus nucleotide data reported in this paper are available from the GenBank™ database under the accession number: MF356556.

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