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# Microsporidia and *Cryptosporidium* in horses and donkeys in Algeria: Detection of a novel *Cryptosporidium hominis* subtype family (Ik) in a horse

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ABSTRACT

A total of 219 and 124 individual fecal samples of horses and donkeys, respectively, were screened for the presence of *Cryptosporidium* spp., *Encephalitozoon* spp., and *Enterocytozoon bieneusi* DNA by genus-specific nested PCR. Isolates were genotyped by sequence analysis of SSU rRNA, GP60, TRAP-C1, COWP, and HSP70 loci in *Cryptosporidium*, and the ITS region in microsporidia. *Cryptosporidium* spp. was detected on 3/18 horse farms and 1/15 farms where donkeys were kept. Overall, five (2.3%) horse and two (1.6%) donkey specimens were PCR positive for *Cryptosporidium*. Genotyping at SSU and GP60 loci revealed that three isolates from horses and donkeys were *C. parvum* subtype family IIaA16G1R1, one isolate from a horse was, *C. muris* RN66, and one isolate from a donkey was *C. muris* TS03. An isolate from a horse shared 99.4% and 99.3% similarity with *Cryptosporidium hominis* and *C. cuniculus*, respectively, at the SSU locus. This isolate shared 100% identity with *C. hominis* at the TRAP-C1, COWP, and HSP70 loci, and it was from the novel gp60 subtype family IkA15G1.

Microsporidia were found on 6/18 horse and 2/15 donkey farms. *E. bieneusi* was identified in 6.8% (15/219) and 1.6% (2/124), and *Encephalitozoon cuniculi* was identified in 1.8% (4/219) and 1.6% (2/124), of horses and donkeys, respectively. Three genotypes of *E. cuniculi* (I, II and III) were detected in horses, and *E. cuniculi* genotype II was detected in donkeys. Four genotypes of *E. bieneusi* (horse1, horse 2, CZ3, D) were described in horses. An additional five horses and two donkeys were positive for *E. bieneusi*, but the isolated were not genotyped. Neither *Cryptosporidium* nor microsporidia prevalence were affected by sex, age, type of breeding, or whether the host was a horse or a donkey.



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## 1. Introduction

Both horses and donkeys are used worldwide for work, food, and social activities. Both wild and domestic equines are exposed to a complex mixture of multicellular and unicellular parasites. Although most of these parasites are equine-specific, horses and donkeys can be infected by host-nonspecific and zoonotic parasites including *Cryptosporidium* and several microsporidia (Grinberg et al., 2003; Kouam et al., 2010; Santín et al., 2010).

Approximately 1200 species of microsporidia are known, and they infect all major animal groups. Most of these ubiquitous obligate intracellular parasites infect invertebrates and fish, but 14 species in eight genera infect mammals (Didier et al., 1995; Didier and Weiss, 2006). Although equine microsporidiosis is known to cause abortion (Patterson-Kane et al., 2003; Szeredi et al., 2007; van Rensburg et al., 1991), microsporidia in horses remains on the periphery of scientific interest. Three recent reports have described the prevalence of *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* and the course of infection of *E. cuniculi* in horses from Colombia and the Czech Republic (Santín et al., 2010; Wagnerová et al., 2012, 2013).

Protozoans of the genus *Cryptosporidium* are parasites inhabiting the digestive tract and/or respiratory systems of birds, fish, reptiles, and mammals, including equines (Mtambo et al., 1997; Ryan et al., 2003). Twenty five species and more than 40 genotypes of *Cryptosporidium* have been described to date (Ryan and Xiao, 2014), and only three of these – *C. parvum*, *C. erinacei* (previously known as *Cryptosporidium* hedgehog genotype) and *Cryptosporidium* horse genotype – are known to infect horses (Chalmers et al., 2005; Laatamna et al., 2013; Ryan et al., 2003). However, only eight of the 30 studies of horse cryptosporidiosis reported the species/genotype present (Burton et al., 2010; Grinberg et al., 2008, 2003, 2009; Chalmers et al., 2005; Imhasly et al., 2009; Ryan et al., 2003; Veronesi et al., 2010), so the actual number of *Cryptosporidium* taxa infecting horses may be greater.

A number of *Cryptosporidium* spp. (*C. parvum*, *C. erinacei* and *Cryptosporidium* horse genotype) and microsporidia (*Encephalitozoon intestinalis*, *E. cuniculi* genotypes I and II, and *E. bieneusi* – genotypes D, EbpA, G and WL15) detected in horses and donkeys to date have been shown to cause human infection (Didier et al., 2000; Mathis et al., 2005; Nichols et al., 2014). Therefore, there is a need to better understand the zoonotic potential of horse microsporidiosis and cryptosporidiosis. This study examines the prevalence of *Cryptosporidium* and microsporidia in wild and domestic horses and donkeys from Algeria.

## 2. Materials and methods

### 2.1. Origin of samples and animals

The research was performed from November 2011 to May 2013 on 4 horse farms in an urban area and 20 small private farms of horses and donkeys in rural areas located in 4 provinces in Algeria. The farms were selected without previous knowledge of parasitological status, with the exception of one farm (no. 1), where four cases of *Cryptosporidium erinacei* infection (previously known as *Cryptosporidium* hedgehog genotype) were previously reported (Laatamna et al., 2013). Farm 1, situated in province of Tiaret in west Algeria, is a national center of horse breeding that keeps up to 280 horses and serves as a supplier of horses to other farms located in the different provinces of Algeria. The management system at Farm 1 depended on the season: breeding stallions, mares, and foals spent most of the time on the pasture, except during winter, when they were housed in stables. Equestrian centers 2, 3 and 4, focus exclusively on horse breeding, are located in the capital Algiers, and breed approximately 50, 70, and 90 horses, respectively. Horses are used for horseback riding, horseracing, and show jumping and are maintained in individual boxes. Twenty farms in rural areas included 12 private farms in Bourdj Bou Arréridj (B.B.A) province and eight in Setif province. These rural areas are regions of high plateaus that are almost exclusively agricultural, and horses live in close association with cattle, other ruminants, and domestic birds. Donkeys live unrestricted in these areas and are used for transport and work. Both horses and donkeys are on pasture during the entire year, except during winter, when they are primarily housed in stables.

### 2.2. Sample collection

Fecal samples from horses and donkeys were collected directly from the rectum or from the ground immediately after defecation. Specifically, all horse fecal samples from farms 1–3 ($n = 94$), 40 out of 55 horse samples from farm 4, and three donkey samples from farm 15 were collected directly from rectum (Table 1). Each sample was individually placed into a sterile plastic container without fixatives, and transported in an isotherm box to the laboratory. No repeated analyses of the same animals were included in the survey to prevent cumulative prevalence.

### 2.3. DNA isolation and molecular analyses

Total DNA was extracted from 200 mg of feces by bead disruption for 60 s at 5.5 m/s using 0.5 mm glass beads in a FastPrep®24 Instrument (MP Biomedicals, CA, USA) followed by isolation/purification using a commercially available kit in accordance with the manufacturer's instructions (QIAamp® DNA Stool Mini Kit, Qiagen, Hilden, Germany). Purified DNA was stored at −20 °C prior to being used for PCR. Due to the predicted low number of oocysts (<100) in examined samples, a nested PCR was required (Smith, 2008). The nested PCR approach was used to amplify a region of the small subunit of rRNA gene (SSU; ~830 bp; Jiang et al., 2005; Xiao et al., 1999) and 60 kDa glycoprotein (GP60; ~830 bp; Alves et al., 2003) in all samples. In addition, nested PCR amplifying a region of *Cryptosporidium* Oocyst Wall Protein (COWP; ~550 bp; Pedraza-Diaz et al., 2001; Spano et al., 1997), Thrombospondin-Related Adhesive Protein of *Cryptosporidium*-1 (TRAP-C1; ~780 bp; Spano et al., 1998) and Heat Shock Protein (HSP70; ~1950 bp; Sulaiman et al., 2000) were used in case of detection of a novel isolate of *Cryptosporidium*. Negative and positive controls