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Short communication

# Equine cryptosporidial infection associated with *Cryptosporidium* hedgehog genotype in Algeria

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ABSTRACT

Faecal samples from two horse farms in Algeria keeping Arabian, Thoroughbred, and Barb horses were examined for the presence of *Cryptosporidium* in 2010–2011. A total of 138 faecal samples (16 from a farm keeping 50 animals and 122 from a farm with 267 horses) were screened for *Cryptosporidium* spp. infection using molecular tools. DNA was extracted from all samples. Nested PCR was performed to amplify fragments of the SSU rDNA and gp60 genes to determine the presence of *Cryptosporidium* species and genotypes. Sequence analyses of SSU and gp60 genes revealed four animals positive for the presence of subtype XIIIa A22R9 of the *Cryptosporidium* hedgehog genotype. The infections were not associated with diarrhoea. This study reports, for the first time, the occurrence of *Cryptosporidium* in Algeria and the first occurrence of the hedgehog genotype in horses. These findings support the potential role of infected horses in sylvatic–domestic transmission of *Cryptosporidium*.


## 1. Introduction

Protozoa of the genus *Cryptosporidium* are apicomplexan parasites inhabiting the digestive tract and/or respiratory systems of birds, fish, reptiles, and mammals, including humans (Fayer, 2010). Although equine cryptosporidiosis was first reported in the USA in 1978 in six Arabian foals with inherited combined immunodeficiency (Snyder et al., 1978), the prevalence, genotypes and zoonotic potential of *Cryptosporidium* spp. affecting horses remains poorly understood. This is in contrast to general understanding of cryptosporidiosis in ruminants, pigs, and humans. Natural horse cryptosporidiosis has been reported in the USA (e.g. Burton et al., 2010); Canada (e.g. Gajadhar et al., 1985); Brazil (de Souza et al., 2009); New Zealand (e.g. Grinberg et al., 2009); Europe, including Poland (e.g. Majewska et al., 1999), Italy (Veronesi et al., 2010), Czech Republic (e.g. Ryan et al., 2003), Germany (Epe et al., 2004), UK (e.g. Sturdee et al., 2003), and Switzerland (Imhasly et al., 2009); and the Middle East (Mahdi and Ali, 2002). However, only 8 out of 30 reports of horse cryptosporidiosis were supported by molecular data (Burton et al., 2010; Grinberg et al., 2003, 2008, 2009; Chalmers et al., 2005; Imhasly et al., 2009; Ryan et al., 2003; Veronesi et al., 2010).

Horses appear susceptible to at least two cryptosporidia: *Cryptosporidium* horse genotype, which was detected by Ryan et al. (2003) in Przewalski's horse (*Equus ferus przewalskii*), and *Cryptosporidium parvum*, which is a more frequent cause of horse cryptosporidial infection, and

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is occasionally associated with diarrhoea (e.g. Chalmers et al., 2005; Grinberg et al., 2008, 2009; Imhasly et al., 2009; Veronesi et al., 2010). Thus, more thorough investigations are needed to better understand horse cryptosporidiosis and fully assess its zoonotic potential.

The purpose of this study was to investigate the distribution of *Cryptosporidium* species/genotypes in horses from Algeria using molecular tools based on the genus-specific amplification of the small subunit ribosomal RNA gene, and to assess the distribution and diversity of potentially zoonotic subtypes by sequence analyses of a gene encoding a 60-kDa glycoprotein.

## 2. Materials and methods

### 2.1. Origin of samples

The research was performed over a two-year period (2010–2011) on two horse farms in Algeria, which keep different breeds of horses (Pure Arabian, Thoroughbred and Barb), and represent two different management systems (small private farm (A) and large commercial farm (B)). The farms were selected randomly without previous knowledge of horse parasitization. No other farm animals were kept on the studied farms. The first farm (farm A) was an equestrian centre located in the Algiers that bred approximately 50 horses. Horses were maintained in individual boxes under standard hygienic conditions. The second farm (farm B) was located in the province of Tiaret and kept 267 horses. Broodmares were kept in stables or on pasture, depending on the season. During the summer, mares and foals spent the night on pasture. Breeding stallions were stabled regardless of season.

### 2.2. Sample collection and coprological examination

More than 30% of animals were sampled on each farm to evaluate prevalence. Faecal samples were collected directly from the rectum or immediately after defecation by horses. Each sample was placed into an individual sterile plastic container and transported in an isotherm box to the laboratory. Repeated analyses of the same animals were not included in the survey to prevent estimation of cumulative prevalence.

### 2.3. Gene amplification and sequencing

To identify the *Cryptosporidium* sp. or genotype present, genomic DNA was extracted using the QIAamp® DNA Stool Mini Kit (QIAGEN, Hilden, Germany) from all samples (200 mg from each specimen) as described previously (Sak et al., 2008). Nested PCR was used to amplify a partial sequence of the small subunit rRNA (SSU) and *Cryptosporidium* 60-kDa glycoprotein (gp60) genes (Alves et al., 2003; Jiang et al., 2005). All samples were analyzed in duplicate. Negative and positive controls (DNA of *Cryptosporidium suis* for SSU and *Cryptosporidium hominis* for gp60) were included with each PCR amplification. The PCR amplicons were electrophoresed in 2% agarose gels with 0.2 mg/ml ethidium bromide and visualized under ultraviolet light. The secondary PCR products were sequenced in both directions using ABI BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3730XL sequence analyser (Applied Biosystems, Foster City, CA).

**Table 1**
Prevalence of *Cryptosporidium* spp. in mares, stallions and foals on selected farms in Algeria.

| Farm | Category | No. of screened samples | No. of positive samples (%) |
|---|---|---|---|
| A | Mare | 9 | 0 |
| | Stallion | 7 | 0 |
| | Subtotal | 16 | 0 |
| B | Mare | 51 | 3(5.9) |
| | Stallion | 18 | 1(5.5) |
| | Foal | 53 | 0 |
| | Subtotal | 122 | 4(3.3) |
| Total | | 138 | 4(2.9) |

### 2.4. Phylogenetic analyses

The identity of obtained sequences was examined by a BLAST search (www.ncbi.nlm.nih.gov/blast). Alignment adjustments were made manually to remove artificial gaps using BioEdit. Phylogenetic analyses were performed using the software MEGA5. Neighbour joining (NJ) and maximum likelihood (ML) trees were constructed. All ambiguous positions were removed from each sequence pair. The reliability of branches in trees was assessed using bootstrap analysis with 1000 pseudo-replicates, with values above 50% reported. Partial SSU and gp60 sequences recovered from the horses have been submitted to GenBank under the accession numbers KC305643–KC305650.

### 2.5. Statistical analyses

A two-sample test for equality of proportions without Yate's continuity correction was used to assess relationships between *Cryptosporidium* species/genotype and parameters such as age, sex, and farming practices. All computations were performed with R. 15.1.

## 3. Results

An individual faecal sample was taken from 16 horses from the farm A and 122 horses from the farm B. *Cryptosporidium* was not detected in faecal samples from horses on farm A; whereas, 4 of 122 horses from farm B were *Cryptosporidium* positive (Table 1). Analysis of partial sequences of the SSU rRNA gene showed the presence of *Cryptosporidium* hedgehog genotype in all positive horses. SSU sequences formed one cluster in both neighbour-joining and maximum likelihood analyses. Sequences within a cluster shared 100% identity and differed by 1–6 substitution from SSU sequences previously reported from hedgehogs (99.2–99.8% similarity among GQ214082, GQ214078, GQ259137, and GQ259141; data not shown). Neighbour-joining and maximum likelihood trees constructed from gp60 sequences from this study and sequences published in GenBank revealed the presence of *Cryptosporidium* hedgehog genotype belonging to the XIIIa family. In all sequences, 22 serine-encoding TCA nucleotide repeats were followed by 9 repeats of the