



# Common occurrence of zoonotic pathogen *Cryptosporidium meleagridis* in broiler chickens and turkeys in Algeria

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## ABSTRACT

Only a small number of birds have been identified by molecular techniques as having *Cryptosporidium meleagridis*, the third most important species for human cryptosporidiosis. In this study, using PCR-RFLP analysis of the small subunit (SSU) rRNA gene, we examined the ileum of 90 dead chickens from 23 farms and 57 dead turkeys from 16 farms in Algeria for *Cryptosporidium* spp. *C. meleagridis*-positive specimens were subtyped by sequence analysis of the 60 kDa glycoprotein gene. *Cryptosporidium* infection rates were 34% and 44% in chickens and turkeys, respectively, with all positive turkeys (25) and most positive chickens (26/31) having *C. meleagridis*. All *C. meleagridis* specimens belonged to a new subtype family. The frequent occurrence of *C. meleagridis* in chickens and turkeys illustrates the potential for zoonotic transmission of cryptosporidiosis in Algeria.

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## 1. Introduction

Cryptosporidiosis is a zoonosis caused by the protozoa *Cryptosporidium* spp. Two species, *Cryptosporidium hominis* and *Cryptosporidium parvum*, are involved in most human infections, with the latter largely responsible for zoonotic transmission. A third species that is traditionally associated with birds, *Cryptosporidium meleagridis*, is also commonly found in humans in some countries, although

the extent of zoonotic transmission of the species is not clear (Xiao, 2010).

In birds, although *Cryptosporidium* was initially described in chickens in 1929 (Tyzzer, 1929), no species was named until 1955 when the first avian species, *C. meleagridis*, was described in the ileum of turkeys (Slavin, 1955). Later, another species, *Cryptosporidium baileyi*, was described in the ileum, large intestine, cloaca and bursa of Fabricius of chickens (Current et al., 1986), but was shown in subsequent studies that the parasite also induced respiratory and renal disorders (Lindsay et al., 1987, 1990). A third species, *Cryptosporidium galli*, was described in the proventriculus of chickens (Pavlasek, 1999, 2001). There are also several *Cryptosporidium* genotypes designated as avian genotypes I–V with unknown species status (Ryan et al., 2003; Xiao et al., 2004; Ng et al., 2006; Fayer, 2010).

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*C. meleagridis* is the only *Cryptosporidium* species that can infect both birds and mammals (Akiyoshi et al., 2003; Xiao et al., 2004; Ryan, 2010; Chappell et al., 2011). This species is found in both immunocompromised and immunocompetent humans in industrialized and developing countries (Glberman et al., 2001; Essid et al., 2008; Xiao and Feng, 2008) and was responsible for a recent outbreak of gastroenteritis in Japan (Asano et al., 2006). In birds, the clinical signs and lesions caused by this parasite are generally not specific, and the infection can be in concurrence with other enteric pathogens.

The diagnosis of cryptosporidiosis in birds is based mostly on microscopy and histopathological, immunological, and molecular methods (OIE, 2008). Among the molecular methods, polymerase chain reaction (PCR) followed by melting curve analysis, restriction fragments length of polymorphism (RFLP), or DNA sequencing can be used to determine species, genotypes, or subtypes (Xiao, 2010). Several genotypes of *C. meleagridis* have been reported in humans and birds, based on sequence analysis of the small subunit (SSU) rRNA, 70 kDa heat shock protein (HSP70), and 60 kDa glycoprotein (GP60) genes (Glberman et al., 2001). Currently, at least 11 genotypes of *C. meleagridis* have been identified based on sequence analysis of these genes (Abe, 2010). In this study, we examined the occurrence and subtype identity of *C. meleagridis* in the ileum of dead chicken and turkeys in Algiers, Algeria.

## 2. Materials and methods

### 2.1. Specimens

The study was conducted from February 2010 to April 2011 in Algiers, Algeria. Altogether, 147 dead birds (90 broiler chickens and 57 turkeys) from 23 chicken farms and 16 turkey farms were included, with a mean of 4 birds on each farm. A data collection form was used to record the age of animals, farm management practices, clinical signs, and lesions observed at necropsy. A portion (~5 cm) of the ileum was collected from each bird for morphological and histopathological diagnosis of *Cryptosporidium* spp. Another portion of the same length was sent to the Centers for Disease Control and Prevention in Atlanta, Georgia, USA for *Cryptosporidium* genotyping and subtyping. As the birds died of mostly diarrhea, no tissues of the cloaca, bursa of Fabricius, and the respiratory system were examined for *Cryptosporidium* spp. during this investigation.

### 2.2. Morphologic and histopathological detection of *Cryptosporidium*

The ileum was cut open and mucosa scraped with a blade. A smear was made with the scraping, air-dried, fixed with methanol, and stained using the modified Ziehl–Neelsen acid-fast method (OIE, 2008). The smear was examined for *Cryptosporidium* oocysts under a microscope at 400× and 1000× magnifications. A small fragment of the ileum was fixed in buffered formalin. Sections of

4–5 µm were made from the fixed tissue, stained with hematoxylin and eosin (H&E) stain, and examined for *Cryptosporidium* stages under a microscope at 400× and 1000× magnifications (Gharazouglou et al., 2006). No quantitation of *Cryptosporidium* oocysts or developmental stages was attempted.

### 2.3. *Cryptosporidium* detection and species determination

Tissue specimens were washed with distilled water by centrifugation. DNA was extracted from the washed specimens using the Fast DNA® Spin Kit for Soil (MP Biologicals, Solon, OH, USA). *Cryptosporidium* spp. in extracted DNA were detected by nested PCR amplification of a fragment (826–864 bp) of the SSU rRNA gene as described previously (Xiao et al., 1999; Feng et al., 2007). DNA of *C. baileyi* was used as the positive control. *Cryptosporidium* species were determined by restriction analysis of the secondary PCR products with enzymes *SspI* and *VspI* (New England Biolabs, Beverly, MA, USA) (Xiao et al., 1999). Representative PCR products were sequenced to confirm the species identification.

### 2.4. *Cryptosporidium meleagridis* subtyping

Specimens positive for *C. meleagridis* were subtyped by PCR-sequence analysis of the GP60 gene (Glberman et al., 2001). The primer used were AL3531 (5'-ATAGTCTCCGCTGTATTC-3') and AL3821 (5'-AATTCGCACGAAAGATTTCC-3') in primary PCR and AL3819 (5'-AAGGATGTTTCTGTGAG-3') and AL3824 (5'-TGCAACCAAACTGTAC-3') in secondary PCR, which amplified a ~650-bp fragment of the GP60 gene by nested PCR. The secondary PCR products were sequenced to determine *C. meleagridis* subtype families and subtypes, which were named using the established GP60 subtype nomenclature (Sulaiman et al., 2005; Xiao, 2010; Feng et al., 2011).

### 2.5. Sequence analysis

PCR products of the SSU rRNA and GP60 genes were sequenced in both directions on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the secondary primers and the BigDye1 Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems). The sequences obtained were aligned with each other and reference sequences downloaded from the GenBank using ClustalX (<http://www.clustal.org/>). To assess genetic relationship among *C. meleagridis* subtype families, a neighbor-joining tree was generated from the aligned GP60 sequences using the program TreeCon (<http://bioinformatics.psb.ugent.be/software/details/treecon>) based on genetic distances calculated by the Kimura two-parameter model. The GP60 tree was rooted with a sequence of *Cryptosporidium ferret* genotype (GQ121029). The reliability of branches was assessed by bootstrap analysis using 1000 replicates. Unique GP60 sequences generated in this study were submitted to GenBank under accession numbers JX878610–JX878614.

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