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Cryptosporidium avium n. sp. (Apicomplexa: Cryptosporidiidae) in birds

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Abstract

The morphological, biological, and molecular characteristics of *Cryptosporidium* avian genotype V are described, and the species name *Cryptosporidium avium* is proposed to reflect its specificity for birds under natural and experimental conditions. Oocysts of *C. avium* measured 5.30–6.90 μm (mean = 6.26 μm) × 4.30–5.50 μm (mean = 4.86 μm) with a length to width ratio of 1.29 (1.14–1.47). Oocysts of *C. avium* obtained from four naturally infected red-crowned parakeets (*Cyanoramphus novaezealandiae*) were infectious for 6-month-old budgerigars (*Melopsittacus undulatus*) and hens (*Gallus gallus f. domestica*). The prepatent periods in both susceptible bird species was 11 days post infection (DPI). The infection intensity of *C. avium* in budgerigars and hens was low, with a maximum intensity of 5,000 oocysts per gram of faeces. Oocysts of *C. avium* were microscopically detected at only 12–16 DPI in hens and 12 DPI in budgerigars, while PCR analyses revealed the presence of specific DNA in faecal samples from 11 to 30 DPI (the conclusion of the experiment). *Cryptosporidium avium* was not infectious for 8-week-old SCID and BALB/c mice (*Mus musculus*). Naturally or experimentally infected birds showed no clinical signs of cryptosporidiosis and no pathology was detected. Developmental stages of *C. avium* were detected in the ileum and caecum using scanning electron microscopy. Phylogenetic analyses based on small subunit rRNA, actin, and heat shock protein 70 gene sequences revealed that *C. avium* is genetically distinct from previously described *Cryptosporidium* species.

Keywords

Cryptosporidium avium; Morphology; Molecular analyses; Transmission studies; *Cryptosporidium* avian genotype V

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1. Introduction

Cryptosporidium parasites belong to the phylum Apicomplexa and infect the gastrointestinal tract of a broad range of vertebrate species (Fayer 2010), causing the diarrheal disease cryptosporidiosis. Currently, around 30 species of *Cryptosporidium* infecting fish, amphibians, reptiles, birds, and mammals are considered to be valid (Kváč et al. 2014a; Liu et al. 2013; Qi et al. 2011). Of these, only three have specificity for birds: *Cryptosporidium meleagridis*, *Cryptosporidium baileyi*, and *Cryptosporidium galli* (Current et al. 1986; Ryan et al. 2003b; Slavin 1955). In addition, 11 *Cryptosporidium* genotypes have been described in more than 30 bird species worldwide, including avian I–V, goose genotypes I–IV, duck genotype, and Euroasian Woodcock genotype (Ryan 2010). Of these, only *C. meleagridis* is known to also infect humans (Alves et al. 2003; Cama et al. 2003; McLauchlin et al. 2000; Xiao and Ryan 2004). Although mammal-specific *Cryptosporidium* species and genotypes are rarely detected in birds, *C. hominis, C. hominis*-like, *C. parvum*, and muskrat genotype I have been reported in faecal samples from Canada geese (*Branta canadensis*) (Graczyk et al. 1998; Jellison et al. 2004, 2009; Zhou et al. 2004).

Natural cryptosporidiosis of birds caused by *C. meleagridis* and *C. galli* affects the gastrointestinal tract and manifests in different degrees of enteritis (Gharagozlou et al. 2006; Ryan et al. 2003b), whereas *C. baileyi* infects many sites, including conjunctiva, nasopharynx, trachea, bronchi, air sac, gut, bursa of Fabricius, kidneys, and urinary tract, and manifests in three clinical forms: respiratory disease, enteritis, and renal disease (Lindsay and Blagburn 1990). Usually only one form of the disease is present in an outbreak (Lindsay and Blagburn 1990). Also *Cryptosporidium* avian genotype III was reported as a possible cause of chronic vomiting in peach-faced lovebirds (*Agapornis roseicollis*) (Makino et al. 2010). Pathogenicity has not been described for other bird-derived *Cryptosporidium* genotypes (Ng et al. 2006).

The redescription of *Cryptosporidium* genotypes as new species requires morphometric studies of oocysts, genetic characterizations, and demonstration of host specificity (natural and, where possible, experimental) (Xiao et al. 2004). These data have thus far been lacking for *Cryptosporidium* genotypes from birds (Ng et al. 2006). The present study aimed to address this deficiency for *Cryptosporidium* avian genotype V, a genotype first reported in cockatiels (*Nymphicus holandicus*) in Japan (Abe and Makino 2010) and subsequently in many other bird hosts (Table 1). Based on the collective data from this and other studies, we conclude that *Cryptosporidium* avian V is genetically and biologically distinct from recognized *Cryptosporidium* species, and we propose that it be named *Cryptosporidium avium*.

2. Materials and methods

2.1. Source of oocysts for studies

Oocysts of *C. avium* were originally isolated from faecal samples of four naturally infected adult red-crowned parakeets (*Cyanoramphus novaezealandiae*), which were caged by a private owner in České Budějovice (Czech Republic). *Cryptosporidium avium* oocysts from

these red-crowned parakeets were pooled and used to infect a single 6-month-old hen (hen 1; *Gallus gallus f. domestica*). Oocysts from hen 1 were used to infect other animals (see 2.6.).

2.2. Parasitological examination and oocyst preparation

Animal faeces were screened for *Cryptosporidium* oocysts using faecal smears stained with aniline-carbolmethyl violet (ACMV) (Miláček and Vítovec 1985). Faecal specimens were collected daily and stored in a 2.5% potassium dichromate solution at 4–8°C.

Cryptosporidium oocysts originated from red-crowned parakeets and from hen 1 were purified using caesium chloride gradient centrifugation for morphometry analyses and transmission studies (Kilani and Sekla 1987). The viability of oocysts was examined using propidium iodide (PI) staining by a modified assay of Sauch et al. (1991). Briefly, examined oocysts were washed in distilled water (DW; 10,000 oocysts in 100 μl) and mixed with 1 μl of PI (1% solution, SIGMA). After 30 min of incubation at room temperature in the dark, the oocysts were washed twice with DW. Oocyst viability was examined using fluorescence microscopy (filter 420 nm, Olympus IX70). Oocysts with red fluorescence were considered to be dead, and those without fluorescence were considered viable.

2.3. Oocyst morphology

Cryptosporidium avium oocysts for morphology and morphometry analyses were examined using differential interference contrast (DIC) microscopy, brightfield microscopy following ACMV staining, and fluorescence microscopy following labelling with genus-specific FITC-conjugated antibodies (*Cryptosporidium* IF Test, Crypto cel, Medac) (Olympus IX70 microscope, filter 520 nm). Morphology and morphometry were determined using digital analysis of images (M.I.C. Quick Photo Pro v.3.0 soft-ware; Optical Service, Czech Republic) collected using a Camedia C 5060 WIDEZOOM 5.1 megapixel digital camera (Optical Service). A 20 μl aliquot containing ~10,000 purified oocysts was examined for each measurement. Length and width of oocysts (n = 100) were measured under DIC at 1000× magnification, and these were used to calculate the length-to-width ratio of each oocyst. As a control, the morphometry of *C. baileyi* (n=100) from a naturally infected adult Common Peafowl (*Pavo cristatus*) were measured by the same person using the same microscope. Photomicrographs of *C. avium* (avian genotype V) oocysts observed by DIC, ACMV and IFA were deposited as a phototype at the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Czech Republic.

2.4. DNA extraction and molecular analyses

Total DNA was extracted from 200 mg of faeces, 10,000 purified oocysts, or 200 mg of tissue 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). DNA was isolated and purified using a commercially available kit in accordance with the manufacturer's instructions (QIAamp® DNA Stool Mini Kit or DNeasy® Blood & Tissue Kit, Qiagen, Hilden, Germany). Purified DNA was stored at −20 °C prior to being used for PCR. A nested PCR approach was used to amplify a region of the SSU (~830 bp; Jiang et al. 2005; Xiao et al. 1999), actin (~1066 bp; Sulaiman et al. 2002), and HSP70 genes (~1950 bp; Sulaiman et al. 2000). Both primary and secondary PCR reactions were carried out in a volume of 50 μl; the primary reaction

contained 2 μl of genomic DNA (or water as a negative control) and the secondary reaction contained 2 μl of the primary reaction as template. DNA of *C. parvum* and *C. baileyi* were used as positive controls. Secondary PCR products were detected by agarose gel (2%) electrophoresis, visualized by ethidium bromide staining, and extracted using QIAquick® Gel Extraction Kit (Qiagen). Purified secondary products were sequenced in both directions with an ABI 3130 genetic analyser (Applied Biosystems, Foster City, CA) using the secondary PCR primers and the BigDye1 Terminator V3.1 cycle sequencing kit (Applied Biosystems, Foster City, California) in 10 μl reactions.

2.5. Phylogenetic analyses

The nucleotide sequences of each gene obtained in this study were edited using the ChromasPro 1.7.5 software (Technelysium, Pty, Ltd.) and aligned with each other and with reference sequences from GenBank using MAFFT version 7 online server with automatic selection of alignment mode (http://mafft.cbrc.jp/alignment/software/). Phylogenetic analyses were performed and best DNA/Protein phylogeny models were selected using the MEGA6 software (Guindon and Gascuel 2003; Tamura et al. 2011). Phylogenetic trees were inferred by the maximum likelihood (ML) method, with the substitution model that best fit the alignment selected using the Bayesian information criterion. The Tamura 3-parameter model (Tamura 1992) was selected for SSU and HSP70 alignments, and the general time reversible model (Tavaré 1986) was selected for actin alignment. Bootstrap support for branching was based on 1000 replications. Phylograms were drawn using the MEGA6 and were manually adjusted using CorelDrawX7. Sequences of SSU, actin, and HSP70 derived in this study have been deposited in GenBank under accession numbers KU058875–KU058886.

2.6. Transmission studies

2.6.1. Animals—Three 8-week-old SCID mice (strain C.B-17), three 8-week-old BALB/c mice (Charles River, Germany), three 6-month-old hens (hen 2-4; *Gallus gallus f. domestica*), and three 6-month-old budgerigars (bud 1-3; *Melopsittacus undulatus*) were used for experimental infection studies. In addition, three animals from each host species/strain were used as negative control.

2.6.2. Experimental design—To prevent environmental contamination with oocysts, laboratory rodents were housed in plastic cages and supplied with a sterilized diet (TOP-VELAZ, Prague, Czech Republic) and sterilized water *ad libitum*. Hens and budgerigars were kept in species-appropriate birdcages with sterilized wood-chip bedding and without bedding, respectively, and were supplied with sterilized food and water *ad libitum*. Each animal was inoculated orally by stomach tube with 100,000 purified viable oocysts suspended in 200 μl of distilled water. Animals serving as negative controls were inoculated orally by stomach tube with 200 μl of distilled water. Faecal samples from all animals were screened daily for the presence of *Cryptosporidium* oocyst using ACMV staining and the presence of *Cryptosporidium* specific DNA was confirmed using nested PCR targeting the SSU gene. All experiments were terminated 30 days post infection (DPI). Infection intensity was reported as the number of oocysts per gram (OPG) of faeces as previously described by Kváč et al. (2007). In addition, faecal consistency and colour and general health status was

examined daily. One *Cryptosporidium avium* positive animal from each host group was euthanized 20 DPI. Tissue specimens were processed for PCR detection, histology, and electron microscopy.

2.6.3. Histopathological examinations—The complete examination of all gastrointestinal organs was conducted at necropsy. Tissue specimens from the stomach, small intestine, and large intestine (the entire tract was divided into 1 cm sections) were sampled and processed for histology according to Kváč and Vítovec (2003) and for PCR analyses (see Section 2.3). Histology sections were stained with haematoxylin and eosin (HE), Wolbach's modified Giemsa stain, and genus-specific FITC conjugated monoclonal antibodies targeting *Cryptosporidium* oocyst wall antigens (*Cryptosporidium* IF Test, Crypto cel, Medac).

2.6.4. Scanning electron microscopy—Samples of intestinal tissue originating from a host confirmed to be infected with *C. avium* were fixed in freshly prepared 3% glutaraldehyde (v/v) in cacodylate buffer (0.1 M, pH 7.4) at 4°C and further processed for SEM as described in Valigurová et al. (2008). All samples were examined by JEOL JSM-7401F.

2.6.5. Animal care—Animal caretakers wore new disposable coveralls, shoe covers, and gloves every time they entered the experimental room. All wood-chip bedding, faeces, and disposable protective clothing were sealed in plastic bags, removed from the experimental room, and incinerated. All housing, feeding, and experimental procedures were conducted under protocols approved by the Institute of Parasitology, Biology Centre and Central Commission for Animal Welfare, Czech Republic (Protocols No. 071/2010 and 114/2013).

3. Results

In the present study, *Cryptosporidium avium* was detected in naturally infected red-crowned parakeets (*Cyanoramphus novaezealandiae*) (n=4), which continuously shed oocysts for more than 5 months.

3.1. Oocyst morphology

Oocysts of *C. avium* originated from naturally infected red-crowned parakeets were morphometrically identical to those recovered from experimentally infected hen no. 1, measuring 5.30–6.90 μm (mean = 6.26 μm) × 4.30–5.50 μm (mean = 4.86 μm) with a length to width ratio of 1.29 (1.14–1.47) (n = 100; Fig. 1a), and they were smaller than oocysts of *C. baileyi*, measuring 5.90–7.60 μm (mean = 6.90 μm) × 4.30–6.60 μm (mean = 5.50 μm) with a length to width ratio of 1.25 (1.06–1.43) (n = 100; Fig. 1). Oocysts in faecal smears showed typical *Cryptosporidium* ACMV staining characteristics (Fig. 1b). Fixed *C. avium* oocysts labelled with FITC conjugated anti-*Cryptosporidium* oocyst wall antibody and examined by fluorescence microscopy had typical apple green, halo-like fluorescence (Fig. 1c).

3.2. Molecular characterization

At the SSU locus, all isolates of *C. avium* (from naturally infected red-crowned parakeets and experimentally infected hens and budgerigars) shared 100% identity with each other and with *Cryptosporidium* avian genotype V obtained from cockatiels in Japan (AB471646, AB471647) and China (HM116381). At the actin locus, *C. avium* isolates from all experimentally susceptible hosts shared 100% identity with each other each and with the GenBank sequences of *Cryptosporidium* avian genotype V obtained from cockatiels in Japan (AB471660, AB471661) and China (JQ320301). At HSP70 locus, all sequences of *C. avium* isolates were identical to sequences obtained from a cockatiel (AB471665) and from a rosy-faced lovebird (*Agapornis roseicollis*; AB538401) in Japan. Maximum likelihood trees inferred from sequences of individual genes (data no shown) and concatenated SSU, actin, and HSP70 sequences (Fig. 2) showed that *C. avium* is most closely related to *Cryptosporidium* avian genotype II and also clusters with *Cryptosporidium* avian genotype I and *C. baileyi*.

3.3. Experimental transmission studies

Oocyst used for experimental infections had >90% viability, determined by PI staining. Experimentally inoculated SCID and BALB/c mice did not produce detectable *C. avium* oocysts by microscopy or specific DNA by PCR in faecal samples within 30 DPI. No clinical signs of cryptosporidiosis were detected in any laboratory rodent. Histological and molecular examination of gastrointestinal tract tissue from these rodents did not reveal the presence of *Cryptosporidium* developmental stages or *Cryptosporidium*-specific DNA.

Cryptosporidium avium was fully infectious for all hens and budgerigars. Oocysts were microscopically detected by 12 DPI in both hens and budgerigars. Oocysts of *C. avium* were microscopically detected 12–16 DPI in hens and 12 DPI in budgerigars. The infection intensity of *C. avium* in hens and budgerigars was generally low – hens shed oocysts in range 2,000 to 5,000 OPG, while budgerigars did not shed more than 2,000 OPG. Specific DNA of *C. avium* was detected in faeces of both hens and budgerigars from 11 DPI and then intermittently until the end of the experiment. Infected birds showed no symptoms of the disease and hens and budgerigars necropsied at 20 DPI or 30 DPI showed no macroscopic signs of cryptosporidiosis. No developmental stages of *C. avium* were histologically observed in either hens or budgerigars. However, scanning electron microscopy revealed the presence of developmental stages of *C. avium* attached to the microvilli in the ileum and caecum of hens (Fig. 3) and budgerigars. No pathology-associated changes were observed.

3.4. Taxonomic summary

Cryptosporidium avium—Diagnosis: Oocysts are shed fully sporulated. Sporulated oocysts (n=100) measure 5.30–6.90 μm (mean = 6.26 μm) × 4.30–5.50 μm (mean = 4.86 μm) with a length to width ratio of 1.29 (1.14–1.47). Endogenous stages are unknown.

Type host: red-crowned parakeet (*Cyanoramphus novaezealandiae*)

Other natural hosts: rosy-faced lovebird (*Agapornis roseicollis*), Chicken (*Gallus gallus*), blue-fronted Amazon (*Amazona aestiva*), major Mitchell's cockatoo (*Lophochroa*

leadbeateri), cockatiel (*Nymphicus holandicus*), budgerigar (*Melopsittacus undulates*) (Table 1)

Experimental hosts: hen (*Gallus gallus domesticus*), budgerigar (*Melopsittacus undulatus*)

Prepatent period: 11 DPI

Patent period: at least 30 DPI

Type locality: České Budějovice, Czech Republic

Other localities: Brazil, China, Japan, USA

Site of infection: ileum, ceacum (this study), kidney, ureter, and cloaca (Curtiss et al. 2015)

Material deposited: A phototype, description of oocysts, and DNA are deposited at the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic.

DNA sequences: Partial sequences of SSU, actin, and HSP70 genes were submitted to GenBank under the accession numbers KU058875–KU058886.

Etymology: The species name *avium* is derived from the Latin noun “avis” (meaning a bird) according to ICZN Article 11.9.1-3 as a plural in the genitive case, as it appears to be adapted to birds.

Morphological, genetic, and biological data support the establishment of *Cryptosporidium* avian genotype V as a new species. According to ICZN and criteria for naming species we propose the name *Cryptosporidium avium*.

4. Discussion

Avian-adapted *Cryptosporidium* species and genotypes appear to infect a broad range of bird species (Ryan 2010). This is supported by our finding that *C. avium* could be transmitted from parrots, which are in the order Psittaciformes, to hens, which are in the order Galliformes. It is therefore unsurprising that the host range of *C. avium* overlaps that of other avian-adapted *Cryptosporidium,* including the closely related avian genotype II (Abe and Makino 2010). In contrast to *C. meleagridis*, which has been reported in calves, pigs, rabbits, rats, mice, and humans (Akiyoshi et al. 2003; Cama et al. 2003; Darabus and Olariu 2003; Elwin et al. 2012; Huang et al. 2003; O'Donoghue 1995; Xiao and Ryan 2004) there is no evidence that *C. avium* infects non-avian hosts (present study; Kváč et al. 2014b).

Most birds infected with *C. avium*, including experimentally infected hens and budgerigars, showed no clinical signs of cryptosporidiosis (present study; Ng et al. 2006); however, a 7-yr-old Major Mitchell's cockatoo (*Lophochroa leadbeateri*) showed signs of lethargy, anorexia, and cloacal prolapse (Curtiss et al. 2015). *Cryptosporidium avium* was detected in the kidneys, ureter, and cloaca of the Major Mitchell's cockatoo, and developmental stages were found in the ileum and caecum in the present study. This broad tissue tropism is similar

to the genetically related species and genotype, *C. baileyi* and avian genotype II (Nakamura and Meireles 2015).

Until now, the course of *Cryptosporidium* infection in birds has been described only for by *C. meleagridis*, *C. baileyi*, and *C. galli* (Current et al. 1986; Ryan et al. 2003b; Slavin 1955). We have shown that the prepatent period of *C. avium* (12 days) is significantly longer than that of *C. meleagridis* and *C. baileyi* (4–8 days; Hornok et al. 1998; Lindsay et al. 1988; Rhee et al. 1991; Tůmová et al. 2002) and shorter than that of *C. galli* (25 days, Pavlásek 2001). Differences in the prepatent period of *Cryptosporidium* species are not unusual, even for phylogenetically closely related species infecting the same host. For example, *C. bovis* and *C. ryanae* have a same host range (cattle) and share 98% sequence identity at the SSU locus, but *C. ryanae* has a shorter prepatent period (11 days) than *C. bovis* (16 days) (Fayer et al. 2005, 2008).

Although infected birds shed low numbers of *C. avium* oocysts, shedding continued for the duration of experimental infections (30 DPI), and naturally infected red-crowned parakeets continued to shed oocysts for at least 5 months. A several month-long natural infection was previously observed in various passerines naturally infected with the gastric species *C. galli*. The reported duration of *C. baileyi* and *C. meleagridis* infections ranges from 4 to 151 days and 4 to 21 days, respectively, depending on species and age of the host (Bermudez et al. 1988; Sreter et al. 1995; Tůmová et al. 2002; Woodmansee et al. 1988).

Cryptosporidium avium oocysts from this study (5.30–6.90 × 4.30–5.50 μm) are morphometrically indistinguishable from those of *Cryptosporidium* avian genotype V (5.0–6.6 × 4.1–5.2 μm, Qi et al. 2011), similar to those of *Cryptosporidium* avian genotype II (6.0–6.5 × 4.8–6.6 μm, Meireles et al. 2006; Ng et al. 2006; Qi et al. 2011) and *C. baileyi* (6.3 × 4.6 μm, Current et al. 1986), larger than those of *C. meleagridis* (5.0 × 4.3 μm, Slavin 1955), and smaller than those of *C. galli* (8.0–8.5 × 6.2–6.4 μm, Ryan et al. 2003b), *Cryptosporidium* avian III (7.5 × 6.3 μm, Meireles et al. 2006; Ng et al. 2006) and Euroasian woodcock genotype (8.5 × 6.4 μm, Ryan et al. 2003a).

Phylogenetic analyses based on SSU, actin, and HSP70 gene sequences showed that *C. avium* is genetically distinct from known species and is most closely related to *C. bailey* and *Cryptosporidium* avian genotypes I and II.

At the SSU locus, *C. avium* exhibits 1.70% and 0.28% genetic distance from avian genotypes I and II, respectively, and 2.27% genetic distance from *C. baileyi*. At the actin locus, the genetic distance from avian genotypes I and II is 10.8%, 1.86%, respectively, the genetic distance from *C. baileyi* is 11.04%. At the HSP70 locus, *C. avium* exhibits 4.49% and 12.92% genetic distance from avian genotype II and *C. baileyi*, respectively. These differences are comparable to genetic distances of currently accepted species. For example, at the SSU, actin, and HSP70 loci, the respective genetic distances between *C. parvum* and *C. erinacei* is 0.42%, 0.41%, and 0.72%; *C. hominis* and *C. cuniculus* is 1.11%, 0.37%, and 1.65%; and *C. muris* and *C. andersoni* is 0.70%, 3.54%, and 2.21% at SSU, actin, and HSP70 loci, respectively.

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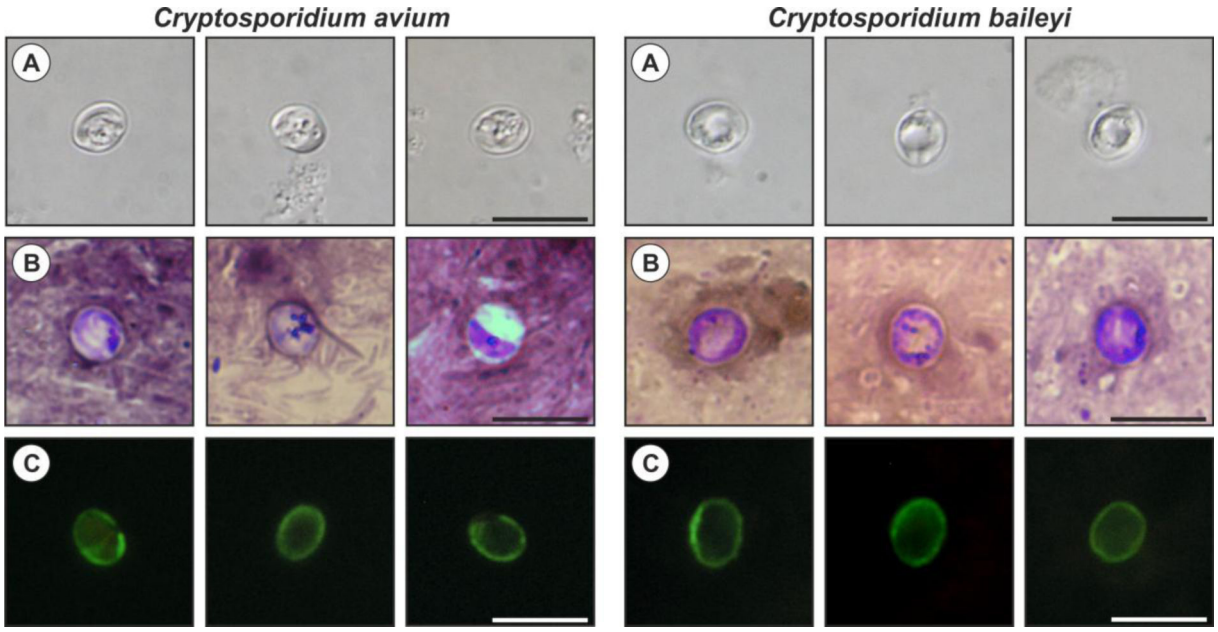

Figure 1.

Cryptosporidium avium and *Cryptosporidium baileyi* oocysts visualized in various preparations: **(A)** differential interference contrast microscopy, **(B)** aniline–carbol–methyl violet staining, and **(C)** labelled with anti-*Cryptosporidium* FITC-conjugated antibody. Bar = 10 μm.

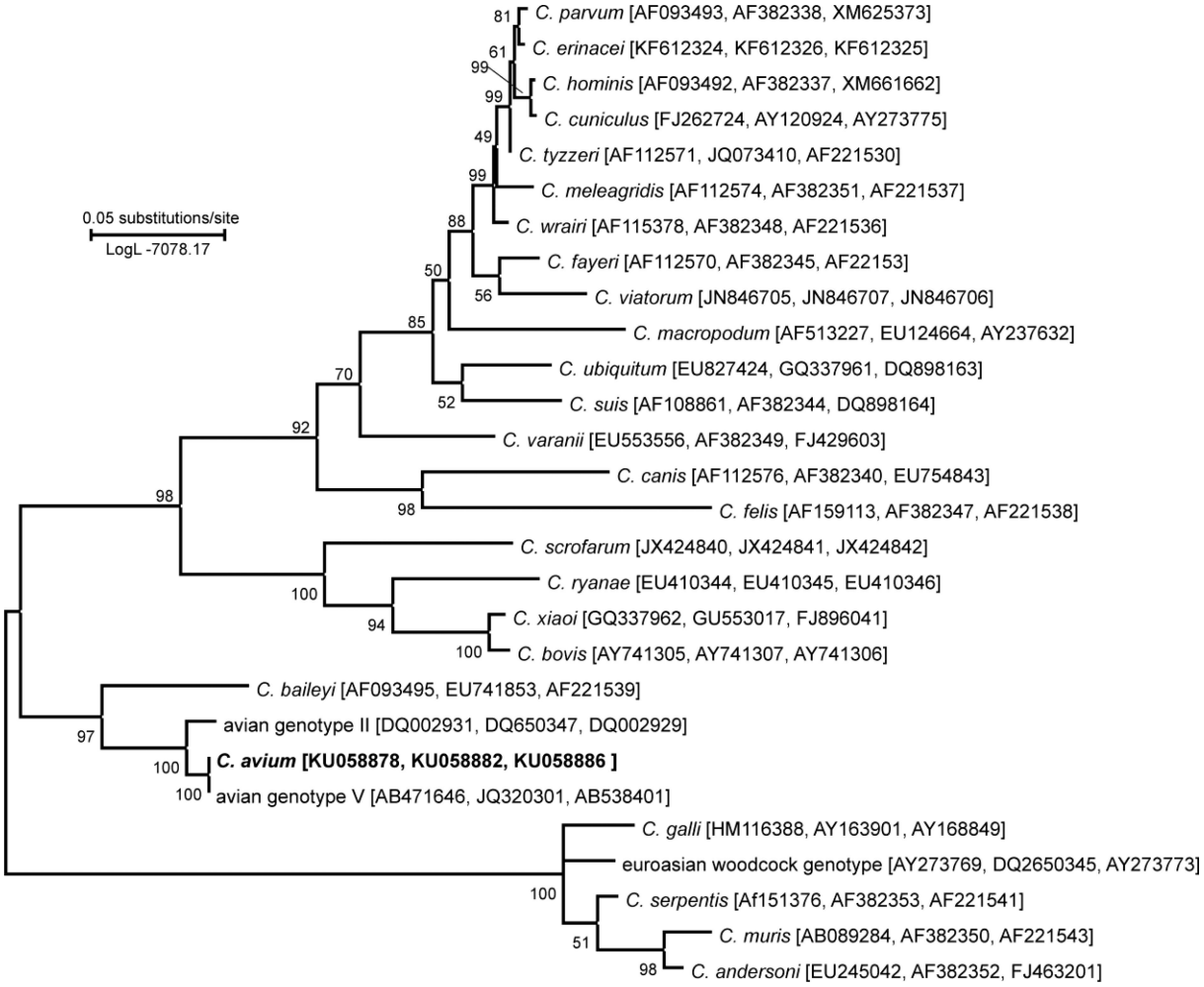

Figure 2.

Phylogenetic relationships between *Cryptosporidium avium* and selected *Cryptosporidium* spp. as inferred by a maximum likelihood (ML) analysis of concatenated sequences constructed from partial DNA sequences of SSU, actin, and HSP70 loci (1234 base positions in the final dataset; model Tamura 3-parametr G+I). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). Numbers at the nodes represent bootstrap values for the nodes gaining more than 50% support. Scale bar included in tree.

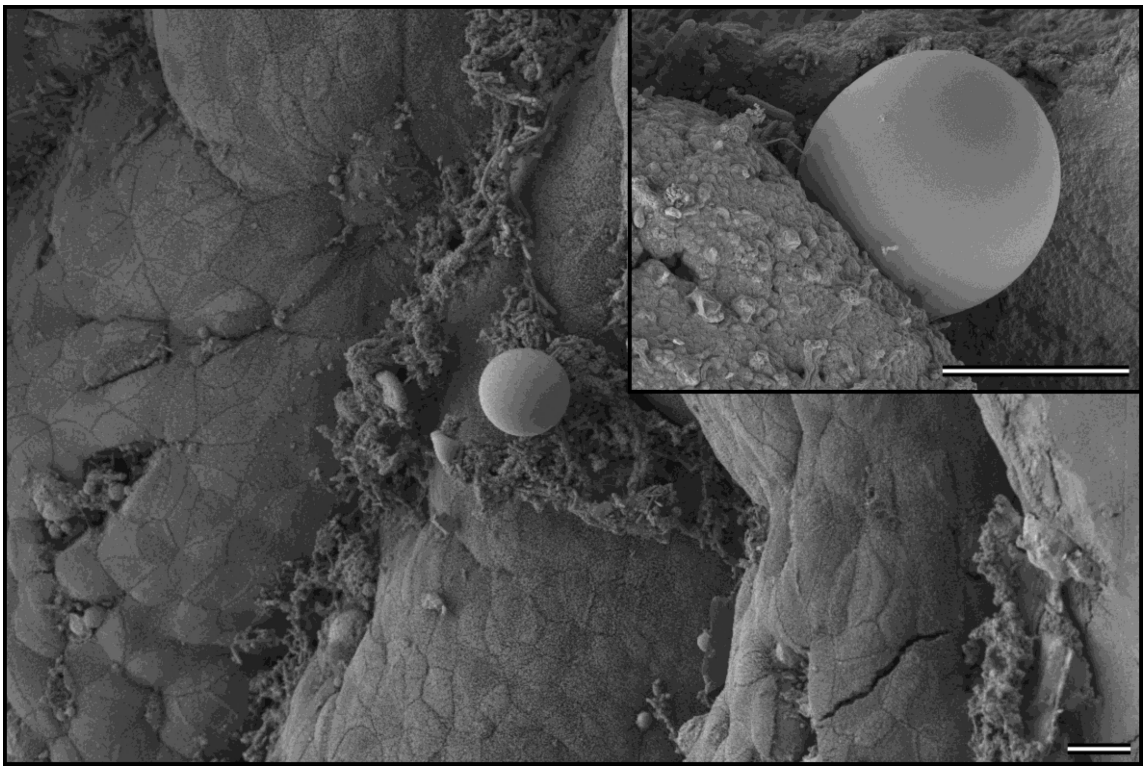

Figure 3.
Scanning electron photomicrograph. Epithelium of ileum of a hen, sacrificed 20 days DPI, showing attached *Cryptosporidium avium*. Detail of the attached *C. avium* developmental stage is provided in the upper right corner. Bar = 10 μm.

Table 1

Occurrence of *Cryptosporidium avium* n. sp. (previously known as avian genotype V) demonstrated on the basis of partial sequences of SSU, actin, and HSP70 in various bird hosts in the world.

Host (Scientific name)	Location	Genes (GenBank accession number)	References
Cockatiel (*Nymphicus holandicus*)	Japan	SSU (AB471646); actin (AB471660); HSP70 (AB471665)	Abe and Makino (2010)
	China	SSU (HM116381)	Qi et al. (2010)
	China	SSU (JQ246415); actin (JQ320301)	unpublished
	China	SSU (KM267556) *	Zhang et al. (2015)
Chicken (*Gallus gallus*)	China	SSU (JX548299)	Wang et al. (2014)
Blue-fronted Amazon (*Amazona aestiva*)	Brazil	SSU (KJ487974)	Nakamura et al. (2014)
Major Mitchell's Cockatoo (*Lophochroa leadbeateri*)	USA	SSU (KP342400)	Curtiss et al. (2015)
Budgerigar (*Melopsittacus undulates*)	China	SSU (KM267556) *	Zhang et al. (2015)

* Identical GenBank accession number for sequence acquired from two different hosts cockatiel and budgerigar; **SSU** – small ribosomal subunit rRNA; **HSP70** – 70 kDa heat shock protein