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Current opinion

Caryospora neofalconis and other enteroparasites in raptors from Mexico

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ABSTRACT

A coprological survey of enteroparasites in raptors (60 Falconiformes) from Central Mexico is reported. Three samples contained coccidian unsporulated oocysts, one contained *Eimeria* sp., one contained trematode eggs and one contained capillarid and trematode eggs and *Eimeria* sp. After sporulation at the laboratory, oocysts from a *Falco peregrinus* were identified as *Caryospora neofalconis.* The phylogenetic analysis of the *C. neofalconis* (GenBank accession number KT037081) showed a close relationship to the Australian strain RY 2014 isolate 16710 (GenBank accession number KJ634019) of *Caryospora daceloe*, with 99.2% similarity. As far as we are aware, this is the first report of *C. neofalconis* in raptors from Mexico and the Americas.

1. Introduction

Enteroparasites are common in wild and captive raptors, and can become significant pathogens if the bird is subjected to excessive stress or disease. Coccidiosis with clinical signs of anorexia or vomiting is recognized in raptors (Klaphake and Claney, 2005). The genus *Caryospora* (Apicomplexa: Eimeriidae) includes coccidian Protozoa and is the third largest genus in the family Eimeriidae. Among these coccidian is the genus *Caryospora* which infects primarily predatory birds and reptiles (Upton et al., 1986).

At least 25 species of *Caryospora* have been identified from birds worldwide (Yang et al., 2014). Of the species identified in birds, 15 have been identified in raptors: 7 from Europe, 2 from Saudi Arabia, 1 from Russia, and 5 from USA (Upton et al., 1990; Alfaleh et al., 2013; McAllister et al., 2013).

In the present study, enteroparasites in raptors from Mexico were surveyed and *Caryospora neofalconis* oocysts were identified in a peregrine falcon (*Falco peregrinus*). As far as we aware, this is the first report of *C. neofalconis* in raptors from Mexico and from the Americas.

2. Materials and methods

2.1. Animal sampling

A total of 60 fresh fecal samples collected during July, 2014 to January, 2015 from healthy, captive Falconiformes in Central Mexico (Guanajuato, Mexico, and Veracruz States). Samples were from *Accipiter gentilis* (1), *Accipiter cooperii* (1), *Falco cherrug* (6), *Falco femoralis* (1), *Falco pelegrinoides* (2), *F. peregrinus* (26), *Falco sparverius* (2) and *Parabuteo unicinctus* (21). Some birds were being rehabilitated for reintroduction, kept in aviary facilities. During sampling, all the birds were fed chicken, Japanese quail, pigeon, or mouse. Some of the *F. peregrinus* were fed pigeons and some *P. unicinctus* were fed free-living white-sided jackrabbits (*Lepus callotis*).

2.2. Microscopic analysis

Fecal samples were collected in individual plastic tubes, which

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were identified, packed into a cooler and immediately transported to Centro de Investigación y Estudios Avanzados en Salud Animal (CIESA-FMVZ-UAEM). The feces were mixed with a 2.5% potassium dichromate solution ($K_2Cr_2O_7$; SIGMA, St. Louis, MO, USA). Samples with unsporulated coccidian oocysts were placed in a thin layer (5 ml) of $K_2Cr_2O_7$ in Petri dishes, incubated at 23–28 °C and monitored daily, until 70% of oocysts were sporulated. Oocysts were recovered using the Sheather's flotation method with sucrose solution and microscopically examined (Duszynski and Wilber, 1997). For image capture, a digital camera (Nikon DS-Fi2) coupled to a light microscope Nikon, Eclipse 80i (Nikon Corporation, Tokyo, Japan), was used. For oocyst measurements the Nikon NIS Elements Software was used.

2.3. Molecular analysis

Before DNA extraction, oocyst samples were washed three times in InhibitEX® Buffer (QIAGEN, Hilden, Germany) by centrifugation. Subsequently, oocyst pellets were resuspended in InhibitEX® buffer and then sonicated (Sonifier 250, Branson, Emerson Electric Co., Ferguson, MO, USA) in ice in three cycles of 5 s (60% pulsed output; power output 5).

DNA was extracted directly from oocysts and purified by using QIAamp® Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocols. The 18S ribosomal RNA (rRNA) gene was amplified from both unsporulated and sporulated coccidian oocysts by using conditions and primers described by Yang et al. (2012): forward primer EIF1 5′-GCTTGTCTCAAAGATTAAGCC-3’ (previously described by Power et al., 2009), reverse primer EIR3 5′-ATGCATACTCAAAAGATTACC-3’. Products of the first PCR were used as template for a second amplification: forward primer EIF3 5′-CTATGGCTAATACATGCGCAATC- 3′ and the reverse primer EIR3 to obtain a 1399–1407 bp fragment (Yang et al., 2012). For PCR, GoTaq® Flexi DNA Polymerase and dNTP Mix (PROMEGA, Madison, WI, USA) were used. Amplification was performed in 50 μl volumes containing 10 μl of GoTaq® Flexi Buffer (5X), 4 μl $MgCl_2$ Solution (25 mM), 2 μl of PCR Nucleotide Mix (10 mM each dNTP), 2 μl of each primer, 5 μl of DNA template, 24.75 μl of PCR grade water and 0.25 μl of GoTaq® Flexi DNA Polymerase (5 U/μl). The PCR conditions consisted of an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 2 min and a final extension at 72 °C for 10 min. And for EIF3 and EIR3 primers, the amplification reactions consisted of an initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 1.5 min and a final extension at 72 °C for 7 min. The PCR products were analyzed by electrophoresis on a 1.0% (w/v) agarose gel in TBE (1X) buffer (PROMEGA, Madison, WI, USA), stained with ethidium bromide and visualized in a UV transilluminator. The image was captured using a MiniBis Pro photodocumentation system (DNR Bio-Imaging Systems Ltd., Jerusalem, Israel). The PCR products were purified from agarose gel using a Wizard SV Gel and PCR Clean-Up System (PROMEGA, Madison, WI, USA), according to the manufacturer's instructions. Then, the DNA products were visualized on a 1% agarose gel to confirm the purification. The DNA products were quantified using a Q5000 UV–Vis Spectrophotometer (Quawell, San Jose, CA, USA). The sequencing of 18S rRNA gene was performed at Macrogen (Seoul, Republic of Korea) using the Sanger dideoxy DNA terminator sequencing method. A Basic Local Alignment Search Tool (BLAST, National Center for Biotechnology Information, Bethesda, MD, USA) search was performed in GenBank (Altschul et al., 1997). Pairwise comparisons for similarity were performed by the program WATER included in European Molecular Biology Open Software Suite (EMBOSS, The European Bioinformatics Institute, Cambridgeshire, UK) (Rice et al., 2000). The phylogenetic analysis was performed by construction of a multiple alignment, removal of gapped columns, and analysis by the maximum likelihood method (Yang et al., 2014) conducted using MEGA 5.2 (Tamura et al., 2011).

3. Results and discussion

Seven of the 60 examined raptors contained enteroparasites: One *F. peregrinus* shed only trematode eggs (119.8 μm × 77.5 μm) and another *F. peregrinus* shed capillarid (63.5 μm × 31.1 μm) and trematode eggs (128.9 μm × 76.6 μm) and unsporulated coccidian oocysts. After sporulation, oocysts from one *F. pelegrinoides* were identified as *Eimeria* sp., labeled as strain ESV-17. Oocysts from the *P. unicinctus* were ellipsoidal (22.2 μm × 15.9 μm), with a bilayered wall and an oocyst residuum present as a spherical compact mass (2.2 μm); these were identified as *Eimeria* sp. and labeled as strain ESV-9 (Table 1). Oocysts from the *F. peregrinus* were identified as *C. neofalconis* and labeled as strain ESV-19. Sporulated oocysts ($n = 30$) were spherical to subspherical, 26.3 μm length × 23.9 μm width (Fig. 1, Table 2). Santos et al. (2011) reported gastro-intestinal parasites in 9 of 66 Falconiformes and 1 of 8 Strigiformes from an animal conservation center in Mexico (Centro de Investigación y Conservación de Vida Silvestre in Los Reyes La Paz, Mexico State). Eggs of *Capillaria* spp., *Eimeria* spp., trematode eggs and *Trichomonas gallinae* were observed. In the present study, capillarid eggs obtained from an *F. peregrinus* might be *Capillaria* sp. Similarly, trematode eggs obtained from two *F. peregrinus* might be *Neodiplostomum attenuatum*. These parasites are commonly found in birds of prey (Krone and Cooper, 2002; Huffman, 2008).

A sequence for the 18S rRNA gene of the unsporulated coccidian oocysts (ESV-17) from a *F. pelegrinoides* was obtained and was most closely related to *Eimeria acervulina*, an eimerid from the domestic chicken (*Gallus domesticus*), with 93.7% similarity. A sequence for the 18S rRNA gene of the sporulated oocysts (ESV-9), from a *P. unicinctus* was obtained and was most closely related to *E. chinchilla*, an eimerid from the long-tailed chinchilla (*Chinchilla laniger*), with 98.9% similarity. A sequence for the 18S rRNA gene of the strain ESV-19 of *C. neofalconis* was obtained and deposited in GenBank (accession number KT037081). The *C. neofalconis* recovered was most closely related to strain RY 2014 isolate 16710 (Genbank accession number KJ634019) of *Caryospora daceloe*, with

Table 1
Enteroparasites recovered from 7 positive raptors in the survey.

Host	Locality	Parasite
Falco pelegrinoides	Xalapa, Veracruz	*Eimeria* sp. (strain ESV-17)
F. peregrinus	Xalapa, Veracruz	*Caryospora neofalconis* (strain ESV-19)
F. peregrinus	Toluca, México	Capillarid, trematode, *Eimeria* sp.
F. peregrinus	Ecatepec, México	Trematode
Parabuteo unicinctus	Villa del Carbón, México	*Eimeria* sp. (strain ESV-9)
P. unicinctus	Villa del Carbón, México	Unsporulated coccidian oocysts
P. unicinctus	Texcoco, México	Unsporulated coccidian oocysts

99.2% similarity. A phylogenetic analysis (Fig. 2) was performed with the sequence determined in the current study. Selected available sequences of apicomplexan organisms were included in the analysis. The human genotype of *Cryptosporidium parvum* (Genbank accession number AF093491) was used as an outgroup.

In the phylogenetic analysis of the 18S rRNA gene, the *Eimeria* sp. (ESV-9) was clustered into a small mammals genetic lineage (Fig. 2). The *P. unicinctus* that shed this eimerid was fed free-living white-sided jackrabbits (*L. callotis*). The shape of this *Eimeria* spp. ESV-9 was similar to the bilayered, ellipsoidal and elongate shape of most of the *Eimeria* sporulated oocysts from other *Lepus* species (Duszynski and Couch, 2013). This suggests that the *Eimeria* sp. observed in the feces of the *P. unicinctus*, may belong to the white-sided jackrabbits (*L. callotis*) eaten a day previously. The *Eimeria* sp. ESV-17 was clustered into an avian genetic lineage (Fig. 2). The *F. pelegrinoides* from which ESV-17 was obtained was fed pigeons two days prior to the sampling. It is not possible to discriminate between coccidia from pigeons and raptors. Reports of eimerid parasites in raptors in the wild might also include parasites from prey – spurious parasitism.

Members of the genus *Caryospora* are the most important causes of health disorders in falconid birds (Upton et al., 1990). Böer in Germany described *C. neofalconis* for the first time by in 1982 (Upton et al., 1990). A diagnosis of *C. neofalconis* was made in 14 juvenile merlins (*Falco columbarius*) and one juvenile snowy owl (*Nyctea scandiaca*) from breeding facilities in the United Kingdom (Forbes and Simpson, 1997). In a parasitological study that included 430 fecal samples collected from 91 birds of prey in the Falcon Breeding Facility in Milotice in the Czech Republic, *C. neofalconis* was detected in four falcons suffering from diarrhea and lethargy. Subsequently, oocysts of *C. neofalconis* were found in 68 samples collected from 30 birds (Pavlík et al., 1998).

As far as we aware, this is the first report of *C. neofalconis* in raptors from Mexico and from the Americas.

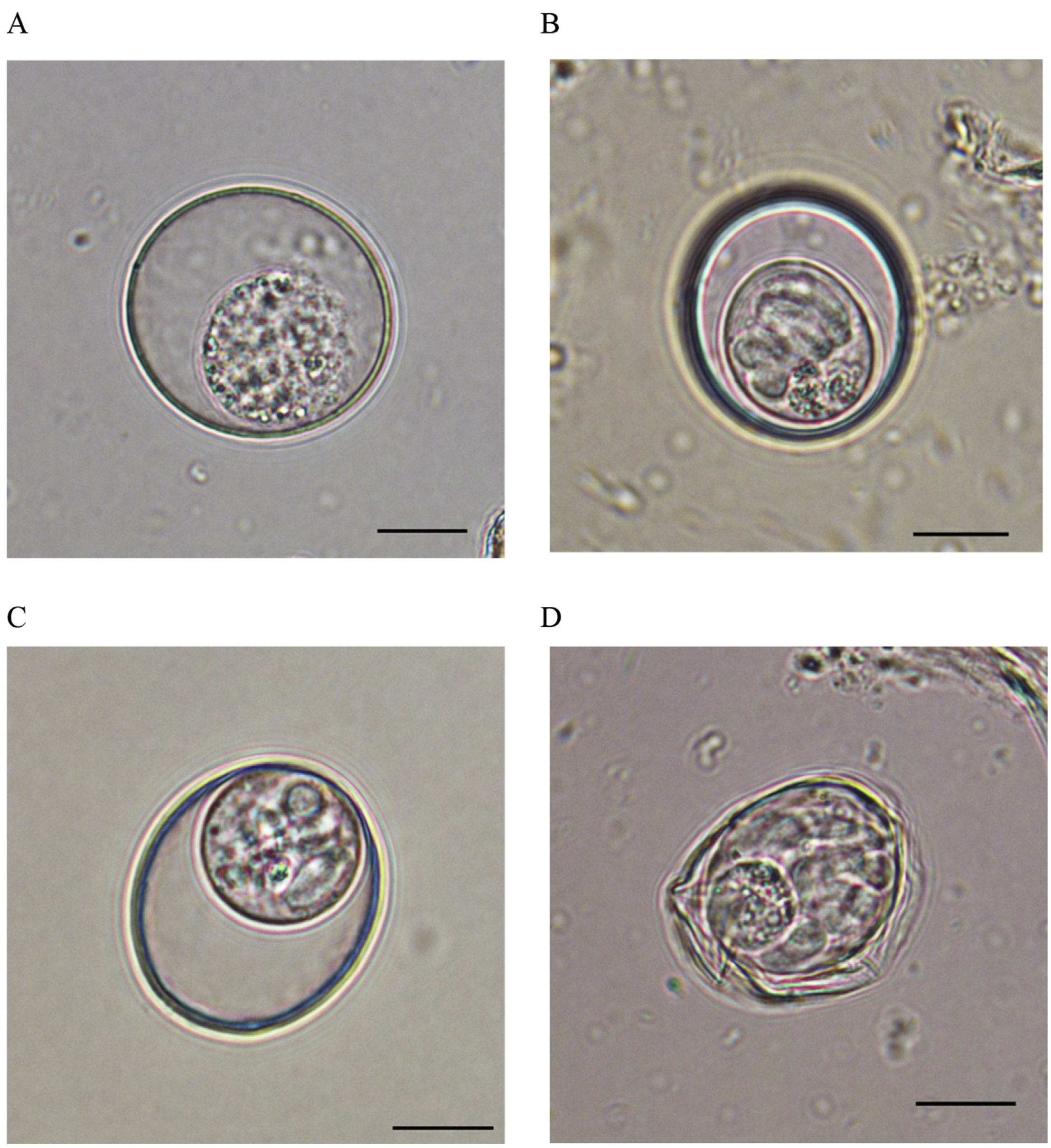

Fig. 1. Photomicrographs of *Caryospora neofalconis* showing one spherical to subspherical unsporulated oocyst (A). Wall bilayered oocysts (B and C) and oocyst residuum present as a spheric diffuse mass (D). Scale bar = 10 μm.

Table 2
Comparative data of *Caryospora* species described from raptors.

Caryospora species	Oocyst shape	Oocyst size (μm)	Sporocyst shape	Sporocyst size (μm)	Locality	Host	Reference
C. aquilae	Subspherical to ellipsoidal	43.0 × 37.5	Spherical to subspherical	23.8 × 23.3	Czech Republic	*Aquila chrysaetos*	Volf et al., 2000
C. biarmicusis	Ovoid	40.2 × 34.7	Spherical	20.1	Saudi Arabia	*Falco biarmicus*	Alyousif et al., 2011
C. boeri	Subspherical	36.6 × 33.4	Ovoid	27.8 × 19.6	Europe	*F. tinnunculus*	Alfaleh et al., 2013
C. cherrughi	Ovoid	32.1 × 29.3	Ellipsoid	24.1 × 19.6	Saudi Arabia	*F. cherrug*	Alfaleh et al., 2013
C. circi	Ovoid	24.5 × 21.8	Spherical to subspherical	16.2 × 15.6	Czech Republic	*Circus aeruginosus*	Volf et al., 2000
C. falconis	Spherical	29.5 × 36.5	Spherical	21.0 × 23.0	Europe	*F. peregrinus*, *F. subbuteo*, *F. tinnunculus*	Alfaleh et al., 2013
C. hanebrinki	Ellipsoidal to ovoidal	48.1 × 42.1	Spherical	24.8	Kansas, USA	*Haliaeetus leucocephalus*	McAllister et al., 2013
C. henryae	Ovoid	41.0 × 37.0	Ovoid	21.6 −25.2 × 19.8 −21.6	Saint Petersburg, Russia	*F. subbuteo*, *F. tinnunculus* *Bubo bubo*	Yakimoff and Matschulsky, 1936
C. kansasensis	Ovoid	37.2 × 32.6	Spherical	22.5	Kansas, USA	*Buteo swainsonii*	Upton et al., 1990
C. kutzeri	Subspherical	38.7 × 34.1	Ovoid	24.6 × 21.0	Europe	*F. biarmicus*, *F. cherrug*, *F. jugger*, *F. mexicanus*	Alfaleh et al., 2013
C. lindsayi	Subspherical	33.7 × 31.6	Spherical	19.2–22.0	Kansas, USA	*Buteo jamaicensis*	Upton et al., 1990
C. megafalconis	Subspherical or ovoid	43.6 × 35.8	Spherical	23.8	Europe	*F. biarmicus*, *F. mexicanus*, *F. peregrinus*, *F. subbuteo*	Alfaleh et al., 2013
C. neofalconis	Subspherical	27.0 × 23.8	Ovoid	18.8 × 14.8	Europe	*F. biarmicus*, *F. mexicanus*, *F. subbuteo*, *F. tinnunculus*, *F. peregrinus*	Upton et al., 1990
C. petersoni	Subspherical	43.1 × 39.8	Subspherical to spherical	23.4 × 23.3	Kansas, USA	*Accipiter striatus*	McAllister et al., 2013
C. uptoni	Spherical or subspherical	28.1 × 26.4	Spherical	18.2 × 17.9	Alabama, USA	*Buteo jamaicensis borealis*, *B. borealis*	Lindsay and Blagburn, 1986, 1989
C. neofalconis	Spherical to subspherical	26.3 × 23.9	Ellipsoid	17.9 × 14.8	Mexico	*F. peregrinus*	This study

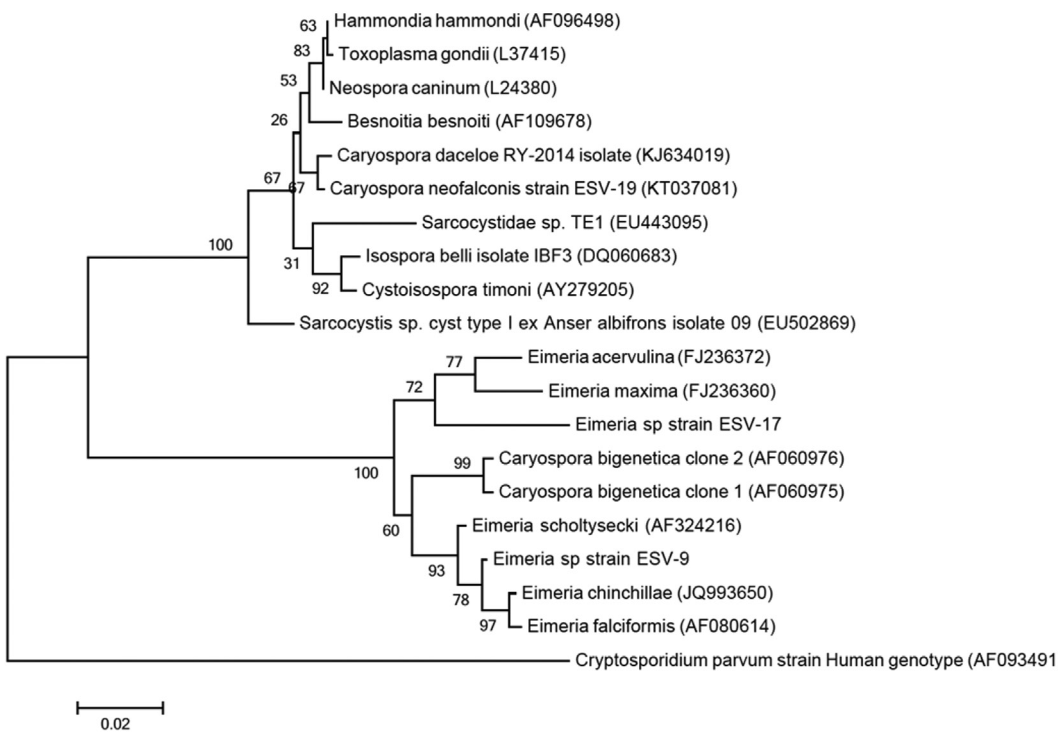

Fig. 2. Phylogenetic relationship of *Caryospora neofalconis*, strain ESV-19, based on maximum likelihood analysis of 18S rRNA gene sequences. The numbers at nodes indicate bootstrap values obtained from 1000 resamplings. The scale bar represents sequence variation.

Conflict of interest

The authors declared that there is no conflict of interest.

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