Current opinion

**Caryospora neofalconis** and other enteroparasites in raptors from Mexico

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**A B S T R A C T**

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 dacelo*, 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.

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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 calottis*).

2.2. Microscopic analysis

Fecal samples were collected in individual plastic tubes, which
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 (K2Cr2O7; SIGMA, St. Louis, MO, USA). Samples with unsporulated coccidian oocysts were placed in a thin layer (5 ml) of K2Cr2O7 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 PCR, GoTaq® (PROMEGA, Madison, WI, USA) were used. Amplification reactions consisted of an initial denaturation at 94 °C for 2 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 MiniBi6 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, Cambridge, 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).

### 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’-GCTTGTCTCAAAATGCATACTCAAAAGATTACC-3’ (previously described by Power et al., 2009), reverse primer EIR3 5’-ATGCATAACTCAAAAGATTACC-3’ gene amplified from both unsporulated and sporulated coccidian oocysts by using conditions and primers described by Yang et al. (2012): forward primer EIF1 5’-GCTTGTCTCAAAATGCATACTCAAAAGATTACC-3’ (previously described by Power et al., 2009), reverse primer EIR3 5’-ATGCATAACTCAAAAGATTACC-3’ (previously described by Power et al., 2009) and the reverse primer EIR3 5’-CTATGGCTAATACATGCGCAATC-3’ (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 MgCl2 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 products were sequenced by the strain ESV-19 isolate 16710 of C. neofalconis (GenBank accession number KT037081). The sequence was submitted to GenBank (accession number KT037081). The C. neofalconis recovered was most closely related to strain ESV-9 (Table 1). Oocysts from the F. peregrinus were identified as C. neofalconis and labeled as strain ESV-9. 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 en 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 attenualum. 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 P. pellegrinoides 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. uncininctus was obtained and was most closely related to E. chinchilla, an eimerid from the long-tailed chinchilla (Chinchilla laniger), with 98.8% 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 daceae, with

### Table 1

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

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. peregrinoides were identified as Eimeria sp., labeled as strain ESV-17. Oocysts from the P. uncininctus 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 en 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 attenualum. These parasites are commonly found in birds of prey (Krone and Cooper, 2002; Huffman, 2008).
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.
Conflct of interest

The authors declared that there is no conflict of interest.

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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 | Alfaileh et al., 2013 |
| C. cherrughi       | Ovoid        | 32.1 × 29.3 | Ellipsoid | 24.1 × 19.6 | Saudi Arabia | F. cherrug | Alfaileh 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 to subspherical | 21.0 × 23.0 | Europe | F. peregrinus, F. subbuteo, F. tinnunculus | Alfaileh et al., 2013 |
| C. heinebrinki     | Spherical 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 | Saint Petersburg, Russia | F. subbuteo, F. tinnunculus | 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 | Alfaileh 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 | Alfaileh 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 boreales, 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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