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Short communication

First detection of *Cryptosporidium parvum* in falcons (*Falconiformes*): Diagnosis, molecular sequencing, therapeutic trial and epidemiological assessment of a possible emerging disease in captive falcons

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ABSTRACT

Cryptosporidiosis in raptors and falcons is well-known to be caused by *Cryptosporidium baileyi* and associated mainly with respiratory pathology. This report presents the diagnosis of an atypical cryptosporidiosis event caused by *Cryptosporidium parvum,* that to the authors' knowledge, is a case observed for the first time in falcons. Two falcons (Gyrfalcon x Peregrine hybrids) were presented for annual check without any clinical signs. Hematology, biochemistry, fecal and crop parasitology, radiographic and endoscopic examinations were performed. Endoscopy revealed microcystic formation of the caudal lung field in the two falcons, adhesions and air sac alterations. Sampling and subsequent cytology revealed fungal spores and acid fast stain organisms (identified as *Cryptosporidium* spp.). Feces and affected lung tissue was further send for *Cryptosporidium* spp.-DNA detection. Fecal samples and lung tissue tested positive for *Cryptosporidium* spp. gp60 gene by PCR. By sequence analysis of the gp60 gene locus, diagnosis of *C. parvum* was confirmed with 100% homology. Despite the fact that falcons didn't recover after 1 month of therapy, eight months after the initial examination they were clinically healthy and had satisfactory flying performance. No other falcons were observed with *C. parvum* infections in the facility so far. The possible source, infection route and implications are discussed.

1. Introduction

Cryptosporidiosis is quite commonly detected in asymptomatic wild birds (Nakamura and Meireles, 2015; Reboredo-Fernandez et al., 2015; Krindges et al., 2013) and currently 7 species and 12 genotypes have been described in 16 avian orders, including the orders of *Cathartiformes, Accipitriformes, Falconiformes* and *Strigiformes* (Ng et al., 2006; Nakamura and Meireles, 2015). More specific, *C. baileyi* was detected in *Cathartiformes* (New world vultures), *Falconiformes* and *Strigiformes* while *C. parvum* was found in *Accipitriformes* (Nakamura and Meireles, 2015). *C. baileyi* has been reported to provoke clinical disease in falcons and owls (scops owl, snowy owl) characterized by upper respiratory signs, central nervous signs, otitis, proventriculitis, and acute death (van Zeeland et al., 2008; Molina-Lopez et al., 2010; Bougiouklis et al., 2013; Nakagun et al., 2017). *C. parvum* has not been found to cause any disease or deaths in raptors and was not detected in *Falconiformes*. So far, it was detected in a sparrowhawk (*Accipiter nisus*), a common buzzard (*Buteo buteo*), a honey buzzard (*Pernis apivorus*) and a black kite (*Milvus migrans*) from Spain (Reboredo-Fernandez et al., 2015). To the authors' knowledge this is the first report of *C. parvum* detection in falcons.

2. Materials and methods

2.1. Clinical presentation and diagnostic investigation

Since its establishment in 1983, more than 20,000 falcons have been treated in Dubai Falcon Hospital (DFH) and around 700 falcons are examined yearly. In May 2016, two intact, female falcon hybrids (gyrfalcon x peregrine falcon) were presented for the first time to receive a routine pre-molting annual health check. Both falcons were captive-bred from a breeding facility in Dubai and kept indoors, tethered individually on a block, together with 15 more falcons. According to the anamnesis the falconer has not reported any problem indicating a

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Table 1
Clinical pathology data of two falcons with *C. parvum* asymptomatic infection.

Parameters	17681 May	17681 June	17682 May	17682 June	Normal Range	Unit
Hematology						
Hb	16.5	19.4	16.2	17.3	12–17	g/dl
PCV	44	52	44	47	34–50	%
WBC	**7.5**	5.8	**8.0**	6.1	2.9–6.3	10^9/l
Heterophiles	**81**	65	**80**	77	48–68	%
Lymphocytes	15	32	17	19	18–38	%
Monocytes	**4**	2	3	3	1–3	%
Eosinophiles	0	1	0	1	0–2	%
Basophiles	0	0	0	0	0–2	%
MCHC	0.38	0.37	0.37	0.37	35–34	g/dl
Biochemistry						
AG Ratio	0.69	0.71	0.80	0.87	1.61–0.63	
ALKP	105	208	121	167	182–583	U/L
Albumin	1.10	1.20	1.20	1.30	0.87–1.20	g/dl
AST	**92**	47	**110**	60	33–81	U/L
Ca	8.30	8.90	8.10	8.60	7–10	mg/dl
GGT	3.00	7.00	3.00	6.00	1–8	U/L
Globulin	1.60	1.70	1.50	1.50	1.40–1.90	g/dl
Glucose	376	385	363	386	220–340	mg/dl
Total protein	2.70	2.90	2.70	2.80	2.30–3.10	g/dl
Uric acid	**10.30**	**10.40**	**11.60**	**10.90**	5.40–8.40	mg/dl

clinical sign of infectious disease for these two or the rest of the falcons kept. The owner reported fluctuation in the flying weight of the birds during the training phase (September to January). Their respective weights on initial presentation were Falcon I/ID 17681–1.310 kg and Falcon II/ID 17682–1.185 kg which is within the normal limits for hybrid falcons. The birds underwent full health check which included detailed physical examination, ancillary ophthalmologic examination, parasitological screening for internal parasites (microscopic examinations of feces and crop swab), hematology, plasma biochemistry, whole body radiography (ventrodorsal and laterolateral views), endoscopic examination (bilaterally caudal thoracic air sacs, crop, trachea, cloaca) and examination of biopsy samples (lung tissue, air sac) taken endoscopically by cytologic examination (Diff-Quick and Ziehl-Neelsen stains), mycotic culture and by aerobic bacterial culture.

2.2. Fecal and tissue DNA extraction

Samples of feces and affected lung tissue were shipped to the Institute of Parasitology, Leipzig University, Germany. DNA was extracted from feces and tissue using Nucleospin®DNA stool kit or Nucleospin®Tissue kit (Macherey-Nagel, Dueren, Germany), respectively, according to the manufacturer's recommendations. The extracted DNA was stored at −20 °C.

2.3. Molecular characterization

To detect *Cryptosporidium* spp.-specific DNA sequences, several PCR reactions were performed targeting conserved 18SrRNA region (Xiao et al., 1999), *Cryptosporidium* outer wall protein (COWP) gene (Spano et al., 1997), and gp60 gene (Alves et al., 2003) of the parasite, respectively. Primers Cryp18S_S1 (TTCTAGAGCTAATACATGCG) and Cryp18S_A1 (CCCATTTCCTTCGAAACAGGA) described before (Xiao et al., 1999) were used to amplify a 1325 bp 18SrRNA gene fragment. PCR conditions were adjusted as follows: a total reaction volume of 25 µL containing 0.5 µM of each primer, 2.5 µL DreamTaq™ Green Buffer (10X, ThermoFisher Scientific), 0.8 mM dNTPs, 0.5 IU Phusion DreamTaq™ polymerase (ThermoFisher Scientific) and 2.5 µL template DNA solution was used. PCR cycling comprised initial denaturation (95 °C for 2 min), 35 amplification cycles (95 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s), and a final extension step (72 °C, 5 min). For the COWP gene, primers cry-9 (ggactgaaatacaggcattatcttg) and cry-15 (gtagataatggaagagattgtg) were employed to amplify a COWP gene fragment of approximately 550 bp length. PCR conditions were similar to 18SrRNA gene amplification except of denaturation time of 30 s, annealing time of 30 s, and elongation period of 90 s during the 35 amplification cycles. A gp60 gene fragment of variable size (approx. 880–1,020 bp) was amplified by primers AL3531 (ATAGTCTCCGCTG TATTC) and AL3535 (GGAAGGAACGATGTATCT) described by Alves et al. (2003). The PCR reaction was performed under similar conditions as described above for 18SrRNA gene by using an annealing temperature of 50 °C during amplification cycles. In this PCR, an additional dilution (1:100 DEPC water) was analyzed for the fecal sample. For all PCR reactions, DNA amplification band patterns were analyzed by gel electrophoresis and subsequent ethidium bromide staining of agarose gels and ultraviolet light visualization. Sequence analyses were performed at the Core Unit DNA technologies, Medical Faculty, Leipzig University.

3. Results

3.1. Clinical results

Wet mount parasitology of crop and feces was negative for motile protozoa, eggs, or parasitic larvae. Hematology and biochemistry revealed mild elevation of the white blood cell counts, heterophilia and mild monocytosis, elevated AST (aspartate aminotransferase) and uric acid in both falcons. The slight elevation of the uric acid was attributed to dehydration and strenuous training. The clinical laboratory results of the two falcons are summarized in Table 1. The radiographic examination was generally unspecific with focal opacities of the caudal lung field and mild splenomegaly. The main finding indicating an infectious process were multiple microcystic-like lesions in ostia and caudal lung field and caudal thoracic air-sacs found endoscopically (Fig. 1). Samples were taken endoscopically and were examined cytologically (Diff Quick and Ziehl-Neelsen stain). The samples were positive for acid fast stained microorganisms resembling morphologically *Cryptosporidium* spp. and showed accumulation of inflammatory cells. Fecal samples were also re-tested positive for the same acid fast stained microorganisms. Bacterial aerobic and mycologic cultures revealed only *Aspergillus fumigatus* in one of the falcons (Falcon I/ID 17681). Samples from lungs and feces were stored in −80 °C until further analysis.