CRYPTOSPORIDIOSIS AMONG BIRDS AND BIRD HANDLERS AT ZOO NEGARA, MALAYSIA

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Abstract. This study was carried out at the Malaysian National Zoo to ascertain, not only the current prevalence rate in the birds, but also to determine the association between cryptosporidiosis in birds and the bird handlers. A total of 116 fecal samples from 71 species of birds were collected from six different locations in Zoo Negara, and eight fecal samples from bird handlers were also sampled. Results showed that the prevalence of *Cryptosporidium* oocysts in birds and bird handlers were 3.4% and 12.5%, respectively. The birds that were positive for cryptosporidiosis were Wreathed Hornbill (*Aceros undulatus*) and Great Currasow (*Crax rubra*) from the aviary, Bushy-crested Hornbill (*Anorrhinus galeritus*) from the bird house, and the Common Peafowl (*Pavo cristatus*) from the lake. Birds at the lake showed the highest percentage (12.5%) of positivity, followed by birds at the aviary (5.4%) and the birdhouse (2.8%). Result of the present study seemed to indicate that cryptosporidiosis might be spreading to other species of birds and to other locations in the zoo, which was not previously documented. This study also suggested the probable association of cryptosporidiosis among birds and their bird handlers. However, conclusions can only be drawn after the confirmation of speciation found in birds and bird handlers through molecular identification.

INTRODUCTION

Cryptosporidium is a ubiquitous enteric protozoan pathogen that infects humans, and domestic and feral animals, worldwide. It is an important causative agent of diarrheal disease in humans, leading to significant morbidity and mortality in both developing and developed countries. Cryptosporidiosis, a disease caused by *Cryptosporidium*, was first described in the ceca of chickens by Tyzzer in 1929 (Sterling and Arrowood, 1978; Current, 1989). Subsequently, a report in 1955 described structurally similar parasites in turkeys, and these parasites were later named *C. meleagridis* (Slavin, 1955). In 1986, Current *et al* (1986) isolated and described an organism from chickens and gave the name *C. baileyi* to the species.

Currently, there are 16 species of *Cryptosporidium* that have been identified as having different morphologies and hosts (Xiao *et al*, 2004). *Cryptosporidium* species that have been described in birds include *C. meleagridis*, *C. baileyi*, and *C. galli* (Awad-el-Kariem *et al*, 1997). Only *C. meleagridis*, which infects turkeys and parrots, is a known threat to humans. Nevertheless, *C. baileyi* is probably the most common avian *Cryptosporidium* species because it is able to infect chickens, turkeys, ducks, cockatiels, quails, and ostriches; whereas, *C. galli*, the latest addition to the family, infects hosts such as finches, domestic chickens, capercaillie, and pine grosbeaks (Xiao *et al*, 2004).

In birds, *Cryptosporidium* sp has not only been reported in chickens (Sterling and Arrowood, 1978), turkeys (Sréter and Varga, 1999), quails, pheasants, peafowl, junglefowl, ducks, geese, parrots, pinches, lovebirds, budgerigars, ostriches (Morgan *et al*, 2001), catercaille, and pine grosbeaks (Xiao *et al*, 2004); but also the wrinkled hornbill found in Malaysia (Rohela *et al*, 2005). Avian cryptosporidiosis can manifest as respiratory (Dhillon *et al*, 1994) and intestinal (Lindsay and Blogbum, 1990) diseases. In some cases, cryptosporidiosis might even manifest as renal disease, which can be fatal (Hoerr *et al*, 1986).

The association between cryptosporidiosis in animals and animal handlers is a topic of interest. In 1981, a case of cryptosporidiosis in an animal handler was reported (CDC,

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1982). The animal handler was a previously healthy 25-year-old male who had symptoms of cryptosporidiosis, such as nausea, low-grade fever, moderate abdominal cramps, anorexia, 5 to 10 watery-frothy bowel movements a day, and constipation. The diagnosis was confirmed by finding *Cryptosporidium* sp oocysts in the feces. Following this report, many similar cases of cryptosporidiosis were reported among animal handlers (Augus, 1983; Graczyk *et al*, 1996).

In 2004, a study on the occurrence of *Cryptosporidium* sp oocysts in the fecal samples of birds in Zoo Negara, Kuala Lumpur was conducted. The study showed 6 species of birds were infected with *Cryptosporidium* (Rohela *et al*, 2005). This present study is an expansion of the previous study. This study tries to ascertain not only the current prevalence rate in the birds but also to determine the association between cryptosporidiosis in birds and the bird handlers.

The main objectives of this study were to reevaluate the occurrence of *Cryptosporidium* sp oocyst in the fecal samples of birds in the Zoo Negara, Kuala Lumpur, Malaysia, and to determine the prevalence of cryptosporidiosis among bird handlers and its association with the occurrence of cryptosporidiosis in birds.

METHODOLOGY

Location of study

The study was carried out in the Kuala Lumpur National Zoo, located at Ulu Kelang, 13 km northeast of Kuala Lumpur. The birds at the National Zoo can be found at various places, such as the aviary, bird house, breeding area, animal show, children's world, and lake. All birds in the locations mentioned are caged except those in the lake. Birds housed in the aviary of various species that ranges from smaller birds, such as Lovebirds, to the larger birds, such as Common Peafowl and Hornbill. The birdhouse and the breeding area are places where the birds are quarantined before being sent to the aviary for visitors to view. They are also the places for the rearing the offspring. Children's world is designed especially for children to have a closer view of domesticated animals and birds, such as macaws and parrots. Birds appearing at the animal show are trained to perform stunts and entertainment activities. Storks and pelicans are the birds commonly found in the lake, where they are free to roam.

Sample collection

A total of 116 feces specimens from 71 species of birds were collected and kept in the fecal containers labeled according to the bird species and location. Fecal samples of birds were collected carefully to avoid any contamination with soil or other contaminants. Collection of fecal specimens and the identification of bird species from their respective locations were carried out in the morning with the assistance of the bird handlers. The specimens were returned to the Department of Parasitology, Faculty of Medicine, University of Malaya, and stored in the cold room at 4°C before processing.

Sixteen fecal containers were distributed to the bird handlers for fecal collection, but only eight containers (50%) were returned. The profile of the bird handlers, such as name, age, race, gender, and the location of work were recorded. All samples were returned to the laboratory to be processed.

Stool examination for *Cryptosporidium* oocysts

Direct smear and formalin ethyl acetate concentration technique were employed for all stool samples from both birds and bird handlers. The smears were stained using the Ziehl-Neelsen (acid-fast) stain. After staining, the slides were examined under 400x. Putative *Cryptosporidium* sp oocysts appeared as bright rose-pink spheres (5 ± 1 μm) on a bluish green background. The positive slides were examined again under 1,000x magnification to confirm the presence of *Cryptosporidium* oocysts. All slides positive for *Cryptosporidium* sp oocysts, using the Ziehl-Neelsen staining method were recorded.

RESULTS

A total of 116 fecal specimens from birds and eight fecal specimens from bird handlers were collected from the Kuala Lumpur National Zoo (Table 1 and Table 3). All specimens were

Table 1
List of birds and positive specimens for *Cryptosporidium* oocysts using Ziehl-Neelsen staining technique.

No	Common Name	Species	Location	No. of specimens taken	No. positive for *Cryptosporidium* oocyst
1	African Grey Parrot	*Psittacus erithacus*	BH	1	-
2	African Ground Hornbill	*Bucorvus leadbeateri*	BH	1	-
3	African Spoonbill	*Platalea alba*	A	1	-
4	All domestic chickens	*Gallus gallus*	CW	2	-
5	Barn Owl	*Tyto alba*	BH,AS	2	-
6	Barred Eagle Owl	*Bubo sumatranus*	CW,AS,BH	3	-
7	Black Hornbill	*Anthracoceros malayanus*	BH	1	-
8	Black Kite	*Mulvus migrans*	BH	1	-
9	Black Swan	*Cygnus atratus*	L	2	-
10	Blue and Yellow Macow	*Ara ararauna*	BH,AS,CW	3	-
11	Blue-breasted Quail	*Coturnix chinensis*	BH	1	-
12	Blyth's Hawk Eagle	*Spizaeus alboniger*	BH	1	-
13	Brahminy Kite	*Haliastur Indus*	BH,AS	3	-
14	Brown Barbet	*Calorhamphus fuliginosus*	BH	1	-
15	Buffty Fish Owl	*Ketupa ketupu*	BH	1	-
16	Bulbul	*Pyenonotus*	CW	1	-
17	Bushy-crested Hornbill	*Anorrhinus galeritus*	BH	1	1
18	California Quail	*Callipela californica*	BH	1	-
19	Cassowary		BH	1	-
20	Changeable Hawk Eagle	*Spizaeus cirrhatus*	AS	1	-
21	Common Peafowl	*Pavo cristatus*	L,AS,CW,	7	1
22	Crested Fireback Pheasant	*Lophura ignita*	BA,BH,A	3	-
23	Crested Serpent Eagle	*Spilornis cheela*	BA	1	-
24	Crestless Fireback Pheasant	*Lophara erythrophthalma*	BA,BH,A	3	-
25	Domestic Duck	*Anas domesticus*	A	1	-
26	Eclectus Parrot	*Eclectus roratus*	AS	1	-
27	Egyptian Goose	*Alopochen aegyptiacus*	L	1	-
28	Emu	*Dromalus novaehollandiea*	A	1	-
29	Golden Pheasant	*Chrysolophus pictus*	A	1	-
30	Great Argus Pheasant	*Argusianus argus*	A,BH,BA	3	-
31	Great Currasow	*Crax rubra*	A	1	1
32	Greater Hornbill	*Bucerros bicornis*	BH,AS,A	6	-
33	Greater Sulfur Crested Cockatoo	*Cacatua galerita*	BH	1	-
34	Green Jungle Fowl	*Gallus varius*	BH	1	-
35	Green-winged Dove	*Chalcophaps indica*	A	1	-
36	Green-winged Macow	*Ara chloroplera*	AS	1	-
37	Helmeted Guineafowl	*Numida meleagris*	A,CW	2	-
38	Hill Myna	*Gracula religiosa*	BH	1	-
39	Java Sparrow	*Padda oryzivora*	CW	1	-
40	Lesser Fish Eagle	*Ichthyophaga humilis*	BH	1	-

Table 1 (Continued)

No	Common Name	Species	Location	No. of specimens taken	No. positive for *Cryptosporidium* oocyst
41	Lesser Sulphur Crested Cockatoo	*Cacatua sulphurea*	CW	1	-
42	Little Corella	*Cacatua sanguinea*	CW	1	-
43	Lovebird	*Agopornis fisheri*	CW,BH,A	4	-
44	Magpie Robin	*Copsychus saularis*	BH	1	-
45	Malayan Peacock-Pheasant	*Polyplectron malacense*	BH,BA	2	-
46	Mandarin Duck	*Aix galericulata*	A	1	-
47	Milky Stork	*Mycteria leucocephala*	A	1	-
48	Moluccan Cockatoo/Salmon-Crested Cockatoo	*Cacatua moluecencis*	AS	1	-
49	Mountain Peacock-Pheasant	*Polyplectron inopination*	BH	1	-
50	Nicobar Pigeon	*Caloenas nicobarica*	A	1	-
51	Oriented Pied Hornbill	*Anthracoceros albirostris*	BH	1	-
52	Ostrich	*Struthio camelus*	Savanna	2	-
53	Painted Stork	*Mycteria cinerea*	L,BH	2	-
54	Pigeon	*Columba*	CW,AS	3	-
55	Pin-tailed Parrot Finch	*Erythuraprasima*	BH	1	-
56	Pink-backed Pelican	*Pelecanus rufescens*	A	1	-
57	Rainbow Lory	*Trichoglossus haematodus*	CW	1	-
58	Red Jungle Fowl	*Gallus gallus*	A	1	-
59	Rhinoceros Hornbill	*Buceros rhinoceros*	BH,A	3	-
60	Scarlet Macow	*Ara macoa*	CW	1	-
61	Spotted Billed Pelican	*Pelecanus rufescens*	L	1	-
62	Silver Pheasant	*Lophura nycthemera*	A,BH,BA	4	-
63	Spotted Wood-Owl	*Strix seloputo*	BH,AS	2	-
64	Storm's Stork	*Ciconia stormi*	L	2	-
65	Swan Goose	*Anser cygnoides*	L	2	-
66	Turkey	*Meleagris gallopavo*	CW	2	-
67	White Bellied Sea Eagle	*Haliaetus leucogaster*	BH,AS	2	-
68	White Cockcatoo	*Cacatua alba*	BH	1	-
69	White Rumped Shama	*Copsychus malabaricus*	BH	1	-
70	Wreathed Hornbill	*Aceros undulatus*	A,BH	2	1
71	Wrinkled Hornbill	*Aceros corrugatus*	A	1	-
		Total		116	4

A= Aviary; AS= Animal Show; BH= Bird House; BA= Breeding Area; CW= Children's World; L= Lake

collected according to their locations (Table 2). By using the Ziehl-Neelsen staining technique, four specimens (3.4%) from birds (Table 1) and one sample (12.5%) from a bird handler (Table 3) were positive. *Cryptosporidium* oocysts were identified as bright rose-pink spheres with the size of 5 ± 1 μm on a bluish green background.

The four positive samples for *Cryptosporidium* oocyst in birds were detected in the Great Currasow (*Crax rubra*) and Wreathed Hornbill (*Aceros undulatus*) (both located at the aviary), Common Peafowl (*Pavo cristatus*) (located at the

Table 2
Percentage of fecal bird specimens positive for *Cryptosporidium* oocysts according to location.

Location	Total no. of fecal specimens collected	No. of specimens positive for *Cryptosporidium*	Percentage positive
Animal show	13	-	0
Aviary	37	2	5.4
Bird house	35	1	2.8
Breeding area	5	-	0
Children's world	18	-	0
Lake	8	1	12.5

Table 3
Biodata of the bird handlers examined for *Cryptosporidium* oocysts.

Name	Age (years)	Gender	Race	Location	Result (present of *Cryptosporidium* oocysts)
J	26	M	Malay	Bird house	Negative
S	27	F	Malay	Bird house	Negative
M K	21	M	Malay	Bird house	Positive
M E	25	M	Malay	Bird house	Negative
Z	19	M	Malay	Bird house	Negative
N B H	20	F	Malay	Children's world	Negative
M Y	52	M	Malay	Children's world	Negative
M A M S	20	M	Malay	Children's world	Negative

lake), and Bushy-crested Hornbill (*Anorrhinus galeritus*) (located at the birdhouse) (Table 1). Only 14.3% (1 out of 7) of the specimens taken from the Common Peafowl, 50% (1 out of 2) of the specimens taken from the Wreathed Hornbills, and 100% (1 out of 1) of the specimen from Bushy-crested Hornbills and Great Currasows were positive for *Cryptosporidium* oocysts. Specimens from the bird handlers showed 12.5% (1 out of 8) was positive with *Cryptosporidium* oocysts (Table 3). Locations that were free from *Cryptosporidium* oocysts were the children's world, animal show, and the breeding area. Birds at the lake showed the highest percentage (12.5%) of positivity, followed by birds at the aviary (5.4%) and birdhouse (2.8%) (Table 2).

DISCUSSION

Cryptosporidiosis affects human and animals, which include reptiles, mammals, birds, amphibians, and fish. Studies have confirmed the presence of *Cryptosporidium* oocysts in pets, and domestic and wild animals (Sterling and Arrowood, 1978; O'Donoghue, 1995; Lim and Ahmad, 2001). Since the first detection of *Cryptosporidium* in birds in 1929 by Tyzzer (Sterling and Arrowood, 1978; Current, 1989), many studies have been conducted to determine the presence of *Cryptosporidium* oocysts in various species of birds. However, there are very few studies that have ascertain the naturally occurring cryptosporidiosis in wild birds.

Prevalence rate of 5.9% to 27.3% have been described in flocks in the United States (Lindsay and Blagburn, 1990).

A previous study carried out in 2004 at Kuala Lumpur National Zoo showed six species of birds were positive, with *Cryptosporidium* oocyst in their feces. The positive species included Wrinkled Hornbill (*Aceros corrugatus*), Great Argus Pheasant (*Argusianus argus*), Black Swan (*Cygnus atratus*), Swan Goose (*Anser cygnoides*), Marabou Stork (*Leptoptilos crumeniferus*), and Moluccan Cockatoo (*Cacatua moluccencis*). These birds were located in the aviary and lake, and the Moluccan Cockatoo was routinely used as a show bird (Rohela *et al*, 2005).

This present study showed a prevalence of 3.4% (4 out of 116) of *Cryptosporidium* sp in bird's feces. The prevalence rate has decreased when compared to the previous study that was conducted two years previously at the same sites. In this study, *Cryptosporidium* oocysts were detected in the feces of the Wreathed Hornbill (*Aceros undulatus*) and Great Currasow (*Crax rubra*) from the aviary, the Bushy-crested Hornbill (*Anorrhinus galeritus*) from the birdhouse, and the Common Peafowl (*Pavo cristatus*) from the lake.

Although the prevalence of *Cryptosporidium* sp in birds at the Kuala Lumpur National Zoo has decreased to 3.4% when compared to the previous study, the birds that were positive in this study were different from the ones that were positive in the previous study. The four types of birds that were positive with *Cryptosporidium* oocysts were not found to be positive before. This finding indicates that *Cryptosporidium* infection might be spreading to other species of birds in the zoo. In both the previous and present studies, birds that were found to be positive for *Cryptosporidium* were all asymptomatic. It is very likely that these birds were not quarantined or separated from the other birds that do not harbor the parasite because they did not present any symptoms thereby facilitating the transmission of *Cryptosporidium* oocysts to the other birds.

In terms of location, a comparison between the two studies showed that the aviary and lake were still contaminated with *Cryptosporidium* oocysts, whereas the location that was positive in 2004, the Animal Show, was negative for *Cryptosporidium* in this present study. Conversely, the location that was previously negative in 2004, the birdhouse, was positive in this present study.

Although there were three locations (*ie*, aviary, birdhouse, and lake) in this present study that were positive for cryptosporidiosis, the probability of birds acquiring cryptosporidiosis might be higher in the lake because birds could be infected, not only through contaminated food or close contact, but also through contaminated water. This postulation is confirmed with the findings of this study whereby the birds at the lake showed the highest percentage of positive samples for *Cryptosporidium* oocysts (12.5%), followed by the birds in the aviary (5.4%) and (2.8%) at the birdhouse. Birds at the animal show, breeding area, and children's world were negative for *Cryptosporidium* oocysts.

There are a few contributing factors to the high prevalence of avian cryptosporidiosis in the lake. Birds at the lake are not caged and therefore are free to mix with other birds or fly to any location. They may possibly fly out of the zoo compound. This will facilitate the transmission of *Cryptosporidium* to other areas inside or outside the zoo compound. The implication of birds in the lake being infected is great because the lake water may be contaminated with feces containing *Cryptosporidium* oocysts. This could be a source of transmission to other birds, animals, and humans who contact the contaminated water. Many studies as well as waterborne outbreaks have implicated contaminated water as a source of cryptosporidiosis (Current, 1989; O'Donoghue, 1995; Xiao *et al*, 2004). The findings of this study warrant future systematic study to investigate the quality of water from the lake in order to determine the association of waterborne cryptosporidiosis in a zoological context.

The occurrence of *Cryptosporidium* oocysts in the aviary is probably due to cross contamination. This is because the birds at the aviary are always being relocated from one cage to another. As an example, an asymptomatic infected bird from cage A might be transferred to cage B. Therefore, if cage A is not washed properly, it might still be contaminated with the feces of the previously infected bird that was transferred. When a new

bird, free of infection is placed in cage A, there is a chance for it to acquire the infection through the contaminated cage. This scenario enables the transmission of *Cryptosporidium* oocysts to other birds if it is not controlled properly.

Considering that the types of birds that were positive in this study are common in the wild (*eg*, wreathed hornbill is a common bird of lowland and hill forest of Borneo and Sumatra). It will be crucial to look also into the possibilities of conducting a similar study on the wild birds in Malaysia, to elucidate the role of wild birds in parasitic transmission.

This study discovered that one of the bird handlers who were stationed at the birdhouse was infected with cryptosporidiosis. This person not only managed the birds in the birdhouse, but he also managed the birds at the aviary and lake. As indicated earlier, all the locations where this bird handler was working were positive with infected birds. Therefore, it is difficult to conclude whether he acquired the infection from, or he was the source of infection to, the birds. This is because of the three avian *Cryptosporidium* sp (*eg*, *C. meleagridis*, *C. baileyi*, and *C. galli*), only *C. meleagridis* can infect both human and birds (Xiao *et al*, 2004). Only with the employment of molecular techniques can this dilemma be determined through speciation or genotyping.

In conclusion, a comparison between a study in 2004, and the present study, conducted in the birds at National Zoo, Kuala Lumpur, highlighted that there was a decrease in the prevalence rate in the birds. However, there seemed to be an indication that cryptosporidiosis might be spreading to other species of birds and to other locations in the zoo that was not previously documented. This study also discovered the possible association of cryptosporidiosis among birds and bird handlers. However, conclusions can only be drawn after the confirmation of speciation found in birds and bird handlers through molecular identification.

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