Prevalence and Genetic Characterization of Cryptosporidium Infection in Java Sparrows (Lonchura oryzivora) in Northern China

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Cryptosporidiosis is a cosmopolitan parasitosis that affects a wide range of hosts including birds. As information concerning Cryptosporidium in birds is limited, the present study examined the prevalence and genotypes of Cryptosporidium in Java sparrows in Beijing and Shangqiu, northern China. Three hundred and fifty fecal samples were collected from Java sparrows (Lonchura oryzivora, 225 white Java sparrows and 125 gray Java sparrows) in Beijing and Shangqiu in October 2015, and the samples were examined by PCR amplification of the small subunit ribosomal RNA (SSU rRNA) gene. The overall Cryptosporidium prevalence is 13.42% (47/350), with 16.44% (37/225) in white Java sparrows and 8.00% (10/125) in gray Java sparrows. Cryptosporidium prevalence was 9.82% (16/163) in Java sparrows from Beijing and 16.58% (31/187) in Java sparrows from Shangqiu. The prevalence of Cryptosporidium in females and males was 40.63% (26/64) and 7.34% (21/286), respectively. The Cryptosporidium prevalence in Java sparrows of different ages varied from 10.47% to 16.33%. Sequence analysis of the SSU rRNA gene revealed that all the samples represented C. baileyi. This is the first report of Cryptosporidium in gray Java sparrows in China, which extend the host range for C. baileyi. These results provide baseline information for further studies of molecular epidemiology and control of Cryptosporidium infection in poultry in China.

1. Introduction

Enteric parasite Cryptosporidium is one of the most important causative agents of gastrointestinal illnesses in humans and animals [1–3]. Food and water contaminated by Cryptosporidium oocysts are the most important resources for human infection [3–5]. Moreover, it can be transmitted to humans by direct contact with infected animals [3, 6]. Human infection with Cryptosporidium may show symptoms of diarrheal illness and some other severe diseases. Cryptosporidium infection in birds may present symptoms of respiratory disease, digestive symptoms, and renal disease [7, 8]. Cryptosporidium infection in birds was firstly recorded in 1929. So far, over 30 avian species have been reported as the hosts of Cryptosporidium in the world [7]. Respiratory symptoms frequently occur in birds, and this infection leads to higher morbidity and mortality in birds, especially in broiler chickens [7]. Recently, more than 17 Cryptosporidium species/genotypes (C. galli, C. baileyi, C. meleagridis, C. hominis, C. parvum, C. muris, Cryptosporidium avian I–V, goose genotypes I–IV, duck genotype, and Eurasian woodcock genotype) have been recorded in birds worldwide [7, 9–13]. Of these, C. hominis, C. parvum, and C. meleagridis are well-known causative agents of human infection [12].
The number of sampled birds is too small (n = 25), which may not reflect the actual prevalence of Cryptosporidium in this bird. Also, no information is available about the prevalence of Cryptosporidium in gray Java sparrows in China. Therefore, we investigated the Cryptosporidium prevalence in Java sparrows (Lonchura oryzivora) in China and assessed its associated risk factors.

2. Materials and Methods

2.1. The Investigated Sites. The survey was conducted in Beijing and Shangqiu cities (two main locations of birds’ production), northern China. Beijing city (39°26’–40°03’N, 115°25’–117°30’E) lies to the south of the Yanshan Mountains with an average altitude of 43.5 m, annual precipitation of 626 mm, and average annual temperature of 12.6°C. Shangqiu city (34°82’–34°45’E, 33°98’–34°80’N) is located in Henan province and has a sub humid warm temperate continental monsoonal climate. The altitude of Shangqiu city ranges from 30 m to 70 m, and the average annual temperature is 14.2°C.

2.2. Collection and Preparation of Samples. A total of 350 fecal samples were collected randomly from 225 white Java sparrows (Lonchura oryzivora) and 125 gray Java sparrows (Lonchura oryzivora), of which 163 were obtained from Beijing and 187 were obtained from Shangqiu from pet shops in 2015. Fecal samples were collected by using aseptic cotton and then filtered (0.3 mm wire mesh), and the filtrate was transferred into a 1.5 mL tube. All the samples were stored at 4°C until used for further analysis. Genomic DNA was extracted from feces using a Stool DNA kit (OMEGA, USA) according to the manufacturer’s instructions. The DNA samples were stored at −20°C until used. Information about species, origin, gender, and age of Java sparrows was obtained and listed in Table 1.

2.3. PCR Amplification. Cryptosporidium species/genotypes were determined by nested PCR amplification of the small subunit ribosomal RNA (SSU rRNA) gene using primers F1 (5′-CCCATTTCCTTCTGAAACAGGA-3′) and R1 (5′-TTCT-TAGAGCTTAATACATGCG-3′) for primary amplification and F2 (5′-AAGGATGGAGAACCCTCCA-3′) and R2 (5′-GGAGGGTTGATTATTTAGATAAG-3′) for secondary amplification [16,17]. PCR reaction (25 μL) was composed of 1x PCR buffer (MgCl2-free), 2 mM MgCl2, 200 μM of each deoxyribonucleoside triphosphate (dNTP), 0.4 μM of each primer, 0.2 U of HotStart Taq DNA polymerase (Takara, Dalian, China), and 2 μL of DNA template. The cycling conditions were 5 min at 95°C for initial denaturation and then 35 cycles of 45 s at 94°C, 45 s at 55°C, and 1 min at 72°C, followed by final extension at 72°C for 10 min. Both positive and negative controls were included in each test. PCR products were observed under UV light after electrophoresis in 1.5% agarose gel containing GoldView™ (Solarbio, Beijing, China).

2.4. Sequencing Analyses. The positive PCR products of Java sparrows were sequenced by GenScript Company (Nanjing, China). Cryptosporidium species were determined by alignment with known reference sequences available in GenBank using BLAST (https://www.ncbi.nlm.nih.gov/BLAST/). Representative nucleotide sequences were deposited in GenBank under accession numbers KY034418–KY034427 and KY962159.

2.5. Statistical Analysis. The variation in Cryptosporidium prevalence (y) of Java sparrows of different geographical location (x1), age (x2), species (x3), and gender (x4) was analyzed by χ2 test using SPSS 19.0. In the multivariable regression analysis, each of these variables was included in the binary logit model as an independent variable. The best model was judged by Fisher’s scoring algorithm. All tests were two-sided, and values of probability (P) < 0.05 were considered statistically significant. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were estimated to explore the strength of the association between Cryptosporidium positivity and the conditions tested.

Table 1: Factors associated with prevalence of Cryptosporidium in Java sparrows in Beijing and Shangqiu, northern China.

| Factor | Category | Number positive | Number tested | Prevalence (%) (95% CI) | OR (95% CI) | P value |
|--------|----------|----------------|---------------|-------------------------|-------------|---------|
| Region | Beijing  | 16             | 163           | 9.82 (5.26–14.38)       | Reference   | 0.06    |
|        | Shangqiu | 31             | 187           | 16.38 (11.25–21.91)     | 0.55 (0.29–1.04) |         |
| Species| White Java sparrow | 37           | 225           | 16.44 (11.60–21.28)     | Reference   | 0.03    |
|        | Gray Java sparrow  | 10            | 125           | 8.00 (3.24–12.76)       | 2.26 (1.08–4.73) |         |
| Sex    | Female   | 26             | 64            | 40.63 (28.60–52.66)     | Reference   | <0.01   |
|        | Male     | 21             | 286           | 7.34 (2.22–12.46)       | 8.63 (4.43–16.84) |         |
| Age    | 3–5 months       | 11            | 92            | 11.95 (5.33–18.57)      | Reference   |         |
|        | 6–8 months       | 25            | 153           | 16.33 (10.47–22.19)     | 0.70 (0.49–2.82) | 0.38    |
|        | >8 months        | 11            | 105           | 10.47 (6.41–16.33)      |             |         |
| Total  |          | 47             | 350           | 13.42 (9.85–16.99)      |             |         |
3. Results and Discussion

Forty-seven out of 350 Java sparrows (13.42%) were tested to be Cryptosporidium-positive by nested PCR amplification of the SSU rRNA gene (Table 1). Cryptosporidium prevalence was 9.82% (16/163) in Beijing and 16.58% (31/187) in Shangqiu (Table 1). The prevalence in white Java sparrows and gray Java sparrows was 16.44% (37/225) and 8.00% (10/125), respectively (Table 1). Moreover, female Java sparrows (40.63%, 26/64) had a statistically higher Cryptosporidium prevalence than males (7.34%, 21/286) (Table 1). The infection rates of Cryptosporidium in the different age ranged from 10.47% to 16.33%, with the highest prevalence in the 6–8-month group (Table 1), but the difference was not statistically significant among age groups ($P > 0.05$). Species and sex of Java sparrows were considered as main risk factors to influence the positive rate significantly.

Analyses of the obtained SSU rRNA sequences indicated that all of the 47 isolates represented C. baileyi. These sequences were deposited in GenBank under accession numbers KY034418-KY034427 and KY962159. Comparative analyses of all the obtained SSU rRNA sequences with that of the two known reference sequences (C. baileyi, GU816040 and C. baileyi, AF262324) indicated that no variation was found among them.

In this study, the prevalence of Cryptosporidium in Java sparrows is 13.42%, which is higher than the 4.86% prevalence in birds in Brazil by PCR [11], 8.1% in white Java sparrows in China by ELISA [2]. Moreover, the Cryptosporidium prevalence of the present study was lower than that in pigeons in Thailand (25%) [18]. Different age distributions, test methods, geological environment conditions, and the breeding density may contribute to the difference of Cryptosporidium prevalence [2, 19].

In the present study, all of the obtained 47 Cryptosporidium isolates belong to C. baileyi. C. baileyi is approximately the most common avian Cryptosporidium [20, 21] because it has been reported in a wide range of avian hosts including chickens, turkeys, ducks, black-billed magpie, common myna, crested lark, Gouldian finch, red-billed leiothrix, mixed-bred falcons, zebra finch, rose-ringed parakeet, white Java sparrow, grey partridge, saffron finch, and ruddy shelducks [7, 12, 20–22].

4. Conclusion

The present study revealed overall Cryptosporidium prevalence of 13.42% (47/350) with 16.44% (37/225) in white Java sparrows and 8.00% (10/125) in gray Java sparrows. This is the first report of C. baileyi in gray Java sparrows in China, which extend the host for C. baileyi. These results provide baseline information for further studies of molecular epidemiology and control of Cryptosporidium infection in poultry in China.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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