Prevalence and Molecular Analysis of Cryptosporidium Spp. Isolated From Wild and Domestic Birds

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Abstract: The purpose of this study was: Initially to revealed the distribution of Cryptosporidium spp. in wild and domestic birds in Al-Qadisiya province in Iraq, secondly to determinate genotypic characterization and phylogenetic analyze based on 18S rRNA sequences of Cryptosporidium which obtained from these birds. A total of 236 fecal specimens from six types of birds were screened microscopically to look for Cryptosporidium oocysts using modified Ziehl–Neelsen stain. Cryptosporidium spp. in 32 microscopy-positive specimens were analyzed genetically through DNA extraction after that DNA was amplified and processed with the small-subunit 18S rRNA gene by using ordinary PCR and then produced DNA was analyze to determinate their sequences. Results showed Cryptosporidium oocysts were observed in 137 (58.1%) specimens of birds' feces. They was 54.5% of the turkey, 57.5% of the domestic chickens, 53.8% of the broiler chickens, 62.5% of the common ducks, 76.7% of the quails and 26.7% of the feral pigeon. Sequencing and further phylogenetic analyses identified Cryptosporidium parvum in all birds in our study, Cryptosporidium baileyi observed in domestic and broiler chickens, quail and feral pigeon ducks, Cryptosporidium meleagridis isolated from turkey and quail, finally the species Cryptosporidium gallion recorded from domestic chickens. Because of a high infection percentages with cryptosporidiosis in domestic and wild birds species. So that we suggesting that our birds in the present study would be considered as biological transporters of Cryptosporidium spp. contributing to environmental dissemination with pathogens which infect human and other animals.

Key words: Cryptosporidium Sp. • Domestic Birds • 18s rRNA of Cryptosporidium

INTRODUCTION

Cryptosporidium spp. are one of the most prevalent protozoan pathogen which have a wide range of variety hosts like: mammalian, reptilian, amphibian and fish, beside of all species of birds [1]. Cryptosporidiosis, a disease caused by Cryptosporidium spp., has been reported in over 170 host species [2, 3] and in more than 30 avian species worldwide [4].

In birds there are three main Cryptosporidium species that have been described namely C. baileyi, C. galli and C. meleagridis [5, 6]. Only C. meleagridis, which infects turkeys and parrots, is a known threat to humans [7, 8]. While, C. baileyis likely the most common avian Cryptosporidium species because its ability to infect many birds like: domestic and caged chickens, turkeys, geese, ducks, feral pigeon, lovebirds, budgerigars, cockatiels, quails and ostriches [6], finaly, C. galli, which discovered recently, infects several hosts such as finches, domestic chickens and pine grosbeaks [9]. In 1929, Tyzzer was record the first description of Cryptosporidium infection in the ceca of chickens [4]. Later, a report in 1955 described structurally similar parasites in turkeys and these parasites were named C. meleagridis [9]. In 1986, an organism from chickens was isolated, described and gave the name C. baileyi [10]. Currently, there are 16 species of Cryptosporidium that have been identified which have a different morphologies and hosts [11, 12]. Several researches refers to infection with another species of Cryptosporidium in birds in addition to the three species mentioned previously. The most important of these species is C. parvum which have found natural infection with it in different wild and pet birds [4, 13].

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Avian cryptosporidiosis can manifest as respiratory form Dhillon et al. [14] and intestinal form Adejinmi and Oke [15]. In some cases, cryptosporidiosis might even manifest as a renal form, which can be fatal [16], also Trampel et al. [17] were described cryptosporidiosis in laying hens in tubule urinary and ureter and they named as "Urinary tract cryptosporidiosis" the stages of evolutionary parasite seen on the apical surface of epithelial cells of the collected tubules urinary and ureters.

The traditional methods of diagnosis of cryptosporidium spp. was confirmed through finding oocysts in the feces [18]. Recently, molecular diagnosis was followed to investigate a new species and genotypes of cryptosporidium in different host [19, 20].

Because of the local studies on Cryptosporidiosis in birds in Iraq were rarely and its risk on human health, economically as caused loss of livestock and there of birds in the dissemination of infection with this parasite, our study aimed to investigate the prevalence of cryptosporidiosis in fecal samples of wild and domestic birds in different regions in Al-Qadisiya province, Iraq and molecular analysis of cryptosporidium species which isolated from birds and define the relationship among species genetically according to 18S rRNA gene.

**MATERIALS AND METHODS**

**Collection of Samples and Examination:** A total of 236 birds were collected from different regions of Al-Qadisiya province between May 2013 to June 2014, birds included six species which are: 22 Turkey (Meleagris gallopava), 60 Quail (Coturnix coturnix), 40 domestic chicken (Gallus gallus domesticus), 52 broiler chicken (Gallus gallus), 32 commeduck (Anas platyrhynchos) and 30 Feral pigeon (Columba livia). Fresh fecal samples which took from birds were examined by used hot modified Zeihl-Nelseen stain [18].

The samples were examined for Cryptosporidium oocysts by light microscopy at x400 magnification. Then the oocysts had been concentrated by Sheather's sugar flotation technique as described by Webster et al. [21]. Cryptosporidium-positive samples were stored in 2.5% potassium dichromate and kept at 4°C until DNA extraction.

**DNA Extraction:** Cryptosporidium oocysts were isolated from positive fecal samples using discontinuous density sucrose gradient centrifugation. Genomic DNA was extracted from the purified oocysts using a Stool Genomic DNA extraction Kit (Bioneer Company, South Korea) in accordance with the manufacturer’s instructions and kept at -20°C until detected by the PCR method.

**Molecular Analysis:** A total of 32 Cryptosporidium-positive samples from studied birds were characterized genetically. Cryptosporidium spp. were genotyped by amplifying an 830bp of the small subunit 18S rRNA gene by direct PCR [9], these samples from different birds were analyzed to identified DNA sequencing by using AB DNA sequencing system [22]. The acquired sequences were submitted to a BLAST search to initially define the species/genotypes and to confirm the high similarity and homology with other known sequences of Cryptosporidium spp. in NCBI-GenBank.

**Cryptosporidium Phylogenetic analysis:** All sequences were multiple-aligned and analyzed by using MEGA6 phylogenetic tree program (http://www.megasoftware.net). And then a neighbor-joining or phylogenetic tree was built.

**RESULTS**

Results revealed through examination of 236 samples from wild and domestic birds belonging to six species of birds under study that the percentage of the overall infection was 58.1%. The results in Table 1. Showed the prevalence of Cryptosporidiosis according to species of birds which revealed the highest infection percentage was in quails (Coturnix coturnix) which reached to 76.7% and the least in feral pigeon (Columba livia) which reached to 26.7%.

Our study recorded four species belong to Cryptosporidium be responsible for intestinal cryptosporidiosis in birds which are: C. parvum, C. meleagridis, C. baileyi and C. galli. In addition to their high occurrence, oocysts were also observed in large numbers in fecal samples collected from the same bird.
The molecular analysis of *Cryptosporidium* species was done through extracting DNA amplified with 18S rRNA by used PCR technique. Results of electrophoresis revealed that DNA bands were 830 bp. and their sequences were determinate by sent DNA product to Bioneer company in South Korea.

Results of DNA sequences by used NCBI-BLAST showed that locally species of parasite, which isolate in this study, give a high identical ratio that was between 99%-100% compared with the global analogous species that recorded in NCBI Genbank for the same gene.

The phylogenetic tree of parasite species was drown by used MEGA6 program, the results of neighboring tree revealed two major branches: First branch include specimens of *C. galli* while the second branch include the other three species (*C. parvu, C. meleagridis, C. baileyi*). Also genetic tree showed presence of two difference strains of *C. parvum* can infected the birds with neighboring ratio between them reached to 83% and the same thing was found for *C. meleagridis* but the neighboring ratio was 65% (Fig. 1). Sequencing and phylogenetic analyses identified *C. parvum* in all birds in

**Table 1: The prevalence of Cryptosporidiosis according to species of birds**

<table>
<thead>
<tr>
<th>Species of birds</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Meleagris gallopava</em></td>
<td>22</td>
<td>12</td>
<td>54.5</td>
</tr>
<tr>
<td><em>Gallus gallus domesticus</em></td>
<td>40</td>
<td>23</td>
<td>57.5</td>
</tr>
<tr>
<td><em>Gallus galleus</em></td>
<td>52</td>
<td>28</td>
<td>53.8</td>
</tr>
<tr>
<td><em>Anas platyrhynchos</em></td>
<td>32</td>
<td>20</td>
<td>62.5</td>
</tr>
<tr>
<td><em>Coturnix coturnix</em></td>
<td>60</td>
<td>46</td>
<td>76.7</td>
</tr>
<tr>
<td><em>Columba livia</em></td>
<td>30</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>Total No.</td>
<td>236</td>
<td>137</td>
<td>58.1</td>
</tr>
</tbody>
</table>
our study, *C. baileyi* observed in domestic and broiler chickens, quail and feral pigeon ducks, *C. meleagridis* isolated from turkey and quail, finally the species *C. galli* only recorded from domestic chickens.

**DISCUSSION**

The local birds in our study showed a higher prevalencerate of *Cryptosporidium* oocysts (58.1%) as compared withother concerned studies, the present study agree with previous researches [5, 15, 24, 25]. Statistical analysis showed no significant differences in infection ratios among turkeys, domestic and broiler chicken and common duck while a significant difference found in quail and feral pigeon compared with the other bird species under study. This variation in the percentages attributed to the different areas and environments which samples were collected from it, as well as the different among studied birds in their sensitivity and resistance to infect with oocysts of parasite, its age [13, 26] and bird management may also contribute to high infection rates such as the following methods of feeding, it may be opening breeding type (Free in the fields) as: domestic chickens, turkeys, common duck in agricultural areas [15, 27], or follow the closed breeding method (Caged inside poultry home), as in the case of broiler chicken and sometimes the presence of small rodents in the pet shops, which could be infected with *C. parvum*, probably could have caused the spread of oocysts in their home [27].

We know that birds play very important role as disseminators of many pathogens [28], *Cryptosporidium* are one of them, our study recorded two important species *C. parvum* and *C. meleagridis* which have ability to infect human and variation mammals [8, 29], so that the detection of *Cryptosporidium* oocysts inwild and domestic birds is very significant because of theirmovement from one source to another and contact withsporulatedocysts by man and livestock lead to disseminate cryptosporidiosis infection beside of environmental contamination with viableoocysts [4, 28]. A huge outbreaks of cryptosporidiosis have been associated withdrinking water in Milwaukee in1994 in which about 403, 000 people wereinfected [26]. Therefore birds may be considered as a biological transporter of *Cryptosporidium* spp. that could contaminate rivers and sea or drinking water [24] thus infection transport to human or other animals.

Four species of *Cryptosporidium* were diagnosed from the studied birds which are:*C.parvum*, *C. meleagridis*, *C. baileyi* and *C. galli*. Our results agreeing with many researches which recorded infection with *Cryptosporidium* spp. in different birds [4, 6, 15, 19, 20]. On the other hand, *C. parvum* was isolated from all sex species of studied birds feces, so this refer to ability to infect a wide range of hosts [4, 12]. The presence of *C. baileyi* was observed in domestic and broiler chickens, quail and feral pigeon, this species is usually found as a parasite in many variety of birds hosts. *C. baileyi* is probably the most common avian Cryptosporidium spp. it reported in more than 17 other avian hosts [4, 11, 30]. While *C. meleagridis* just isolated from turkey and quail and *C. galli* diagnosed from domestic chickens only, these may be belong to its weak host specificity, exposure to infection sources, age of birdsand immunity of birds [26].

Molecular analysis of *Cryptosporidium* spp. isolated from birds depending on 18SrRNA revealed distinguish among species clearly, this gene used as a distinction gene, many studies confirmed this fact [6, 12, 31]. According to molecular characterization and phenotypic differences the genus *C. galli* considerable a distinct species compared with other species based on three genetic sites which are 18Sr RNA, HSP70 and actin locus [20, 32]. Among the species/genotypes, phylogenetic tree showed a big genetic similarity between *C. parvum* and *C. meleagridis* with neighbor-joining reached to 99%-100%. Many researches agreed with our results [28, 33]. *C. meleagridis* was the third most common Cryptosporidium parasite in human [12], *C. meleagridis* have a possible to transfer from infected birds to human [24, 29]. Through phylogenetic tree we observed two genotypes belong to *C. parvum* and *C. meleagridis* can infect birds with neighbor-joining reached to 83% and 65% respectively.

Finally, our study established the presence of four species belong to genus *Cryptosporidium* caused cryptosporidiosis in birds and these birds represent as a biological transporters for *Cryptosporidium* oocysts to human, livestock, poultry and other birds.

**REFERENCES**


