Dominance of Zoonotic Pathogen *Cryptosporidium Meleagridis* in Broiler Chickens in Guangdong, China, Reveals Evidence of Cross-Transmission

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Abstract

Background

Cryptosporidium is one of the most prevalent parasites infecting both birds and mammals. To examine the prevalence of *Cryptosporidium* species and evaluate the public health significance of domestic chickens in Guangdong Province, Southern China, we analyzed 1001 fecal samples collected from 43 intensive broiler chicken farms from six distinct geographical regions between June 2020 and March 2021.

Methods

Individual DNAs were subjected to nested PCR-based amplification and sequencing of the small subunit of the nuclear ribosomal RNA gene (SSU rRNA). The 60 kDa glycoprotein gene (*pgp*60) was performed from all positive SSU rRNA samples to characterise subtypes of *C. meleagridis*.

Results

Cryptosporidium infection rates was found to be 13.2%, comprising with infections with *C. meleagridis* (78/1001, 7.8%), *C. baileyi* (48/1001, 4.8%) and mixed infections (6/1001, 0.6%). Three subtype families were identified, IIIb, IIIe and IIIg. Six subtypes were identified in broiler chickens, including one novel (IIIgA25G3R1a) and five previously reported (IIIbA23G1R1c, IIIbA24G1R1, IIIbA21G1R1a, IIIeA17G2R1 and IIIeA26G2R1). Within these subtypes, five known subtypes were genetically identical to those identified in humans.

Conclusions

This is the first report of *C. meleagridis* in chickens from Guangdong. The frequent occurrence of *C. meleagridis* in domestic chickens and the common *C. meleagridis* subtypes identified both in humans and chickens is of public health significance. Our study indicates that broiler chickens represent a potential zoonotic risk for the transmission of *Cryptosporidium* in this region.

Background

Cryptosporidium is a protozoan parasite that infects a wide range of vertebrate hosts, including humans and birds [1]. In birds, *Cryptosporidium* was first found in gallinaceous birds, and since then, has been reported in more than 30 avian species worldwide [2]. Infection of avian flocks with this parasite can lead to respiratory and gastrointestinal disturbances, depending on the infecting *Cryptosporidium* species [3].

Cryptosporidiosis in birds is usually caused by *C. meleagridis*, *C. baileyi*, *C. galli* and *C. avium* [4–9], and rarely by *C. hominis*, *C. parvum*, *C. muris* and *C. andersoni* [10–13]. *C. baileyi* infects the respiratory system and intestines causing high morbidity and mortality [8], whereas *C. meleagridis and C. galli* infect the gastrointestinal tract causing mild to severe diarrhea. In contrast, *C. avium* is mainly associated with asymptomatic infection [8]. Among these species, *C. meleagridis* is the only species that infects both birds and mammals and considered the third most common species infecting humans [14]. Currently, by genetic diversity analysis of the *C. meleagridis*, some families have been suggested to be spread by the anthroponotic and zoonotic transmission routes [15]. Birds are considered a significant reservoirsfor human cryptosporidiosis infection, although the extent of cross-species transmission of these zoonotic species remains unclear [5].

Guangdong Province, southern China, is particularly rich in domestic poultry producers. The interaction between humans and domestic poultry poses the potential for zoonotic transmission. However, there are no reports of *Cryptosporidium* isolated from commercial broiler chickens in this region to date, with the exception of a study in domestic pigeons [16]. The aim of the present study was to estimate the occurrence, genetic diversity of *Cryptosporidium* species and *C. meleagridis* subtypes, and public health significance of chickens in intensive farms in Guangdong Province.

Methods

A total of 1001 fresh pooled fecal samples from the floor was randomly collected from broiler chickens from 43 medium- to large-sized intensive farms (with 1000–25,000 chickens per farm on average) across six distinct geographical regions (Qingyuan, Maoming, Huizhou, Meizhou, Yangjiang and Shanwei) in Guangdong Province, China, between June 2020 and March 2021 (Fig. 1). Each sample contained 4–5 single fecal deposit droppings from different areas inside the poultry house that were pooled into a single sample. In total, 5-10 samples were collected per farm from broiler flocks comprising 50–100 chickens. All chickens were reared on the flat and were around 90 days old. All samples were collected from apparently healthy flocks. Care was taken to avoid sampling fecal material that had been in contact with the ground. The pooled fecal samples (approximately 50 g) were collected into clean plastic bags, kept in ice boxes, and marked with the region, number and date. Samples were then transported immediately to the laboratory and stored at 4°C. Samples were examined within 24 h of collection.

For genomic DNA extraction, approximately 200 mg of fecal sample was suspended in 100 mL of distilled water and centrifuged at 3, 000 · *g* for 10 min. The process was repeated three times. Genomic DNAs were extracted from individual treated materials using the E.Z.N.A.R® Stool DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) in accordance with the manufacturer's instructions and then frozen at -20°C prior to PCR analysis.

Individual DNAs were subjected to nested PCR-based amplification and sequencing of the small subunit of the nuclear ribosomal RNA gene (SSU rRNA, ~830 bp) [17]. To further determine mixed infections and subtypes of *C. meleagridis*, amplification of the 60 kDa glycoprotein gene (*pgp*60; ~900 bp) was

performed from all positive SSU rRNA samples [15]. PCR was conducted in a 50-μL reaction mixture containing 1× rPCR buffer (Takara Shuzo Co., Ltd., Otsu, Japan), 3.0 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 50 pM of each primer, 1 unit of rTaq DNA polymerase (Takara Shuzo Co., Ltd), 2 μL of DNA sample and 1 μL of bovine serum albumin. Known test-positive (cattle DNA) and test-negative (distilled water) controls were included with each PCR reaction. The amplification products were separated with electrophoresis in 1.5% agarose gels, stained with ethidium bromide and visualized on a UV transilluminator.

All secondary PCR amplicons were sequenced using an ABI PRISM™ 3730 XL DNA Analyser (Applied Biosystems, Foster City, CA, USA) in both directions. Sequences were aligned by the program Clustal X version 2.1 (http://clustal.org/) and adjusted manually by BioEdit 7.04 software (www.mbio.ncsu.edu/BioEdit/bioedit.html). The adjusted sequences were submitted to a BLAST search to initially define the species and to further confirm the high similarity with other known sequences of *Cryptosporidium* spp. in the GenBank database.

Phylogenetic analysis was performed using Bayesian inference (BI) and Monte Carlo Markov Chain (MCMC) methods in MrBayes version 3.2.6 (http://mrbayes. sourceforge.net/). Posterior probabilities of > 0.95 are indicated at all major nodes.

Statistical analysis was performed by chi-square tests and differences were considered significant when $p < 0.01$ was obtained using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated.

Results

Of 1001 broiler chicken DNA samples, 132 samples tested positive by PCR amplification of the SSU rRNA gene, equating to an overall prevalence of *Cryptosporidium* of 13.2%. The PCR-positive chickens were detected on 24 of the 43 farms from the six geographical regions examined, with prevalence ranging from 4.0–62.5% (Additional file 1: Table S1). There was no significant statistical difference in geographical provenance and prevalence for each region ($\chi^2 = 14.209$, df = 5, P = 0.014) (Table 1).

Sequencing of the SSU amplicons ($n = 132$) revealed *C. baileyi* in 48 (4.8%) and *C. meleagridis* in 78 (7.8%). Six mixed-species infections were also detected. Seven distinct SSU rRNA sequences were deposited under GenBank accession numbers OK560460- OK560466. *Cryptosporidium* was detected in all age groups (Table 2), and chickens of 61-90 days of age (17.8%) showed a significantly higher infection rate than chickens of < 30 days (8.3%), 31-60 days (12.0%) and > 90 days (6.8%) of age ($\chi^2 = 12.123$, df = 3, P = 0.007). *C. baileyi* was detected in chickens of all age groups (Table 2), and statistically significant differences were observed between age groups ($\chi^2 = 20.600$, df = 3, P = 0.000), with a higher infection rate for *C. bailey* in 61-90-day-old chickens (8.6%). *C. meleagridis* was only detected in chickens of ≤ 90 days of age, and the infection rates were almost similar across the three age groups ($\chi^2 = 0.092$, df = 2, P = 0.955).

Among the 78 *C. meleagridis*-positive specimens, 64 yielded *gp*60 PCR products of the expected size. Alignment of the *gp*60 nucleotide sequences obtained here and references downloaded from the GenBank database revealed the presence of six subtypes, including five known (IIIbA23G1R1c, IIIbA24G1R1, IIIbA21G1R1a, IIIeA17G2R1 and IIIeA26G2R1) and one previously unreported IIIg25G3R1 variant (IIIbA25G3R1a) (GenBank: OK 562693-562699). As expected, the phylogenetic tree revealed three distinct clusters, representing the three subtype families (IIIb, IIIe and IIIg) (Fig. 1). The most common subtype family, IIIb, was identified in 51 samples, including known subtypes IIIbA23G1R1c (*n* = 24), IIIbA24G1R1 (*n* = 20) and IIIbA21G1R1a (*n* = 7). The second most common subtype family, IIIe, was identified in 11 chicken isolates and comprised three distinct subtypes, two known IIIeA17G2R1 subtypes (*n* = 8) and a known IIIeA26G2R1 subtype (n = 3). Finally, for subtype family IIIg, the IIIgA25G3R1a, was identified in two chicken isolates.

Discussion

To our knowledge, this is the first report of the presence and prevalence of *Cryptosporidium* in intensively farmed chickens in Guangdong Province, although previous studies have reported in Hubei, Zhejiang, Henan and Anhui in China [4, 9, 18, 19]. In our study, the overvall prevalence of *Cryptosporidium* in chickens (13.2%; 132/1001) was comparable to previous values reported for domestic chickens in Brazil (12.6%; 24/190) [20], China (10.2%) [9], Syria (9.9%) [21], higher than Iran (0.5%) [22], Tunisia (4.5%) [23], Jordan (4.8%) [24], and Germany (5.7%) [13], but lower than Brazil (25.6%) [25] and Algeria (34.4%) [5]. Differences in hygiene, management practices, sample origin and detection methods may contribute to these differences in prevalence of *Cryptosporidium* in poultry flocks.

In addition, *Cryptosporidium* infection in broiler chickens appeared to be age-related. However, unlike the age-related infection pattern whereby the infection rate decreases with increasing age of infected animals in ruminants [26], the highest infection rate 17.8% was detected in 61-90-day-old broiler chickens, compared with the other age groups ($p < 0.01$) (Table 2). In a previous study in China, chickens (≤ 4 months) had the highest infection rate [9], which was partially in agreement with our results. It is worth noting that most broiler chickens of 61–90 days of age are sold, therefore oocysts may be disseminated during the process of transfer and new infection may result. These broiler chickens should be considered as important reservoirs of *Cryptosporidium*, although the age-related association should be verified by further research.

C. meleagridis and *C. baileyi* were confirmed by molecular characterization of the SSU rRNA gene, which was consistent with previous studies reported in farmed and wild birds in China, including chickens, domestic pigeons, quails, ducks, ostriches, and white Java sparrows [4, 6, 13, 16, 20, 23, 27, 28]. *C. baileyi*, originally isolated from commercial broiler chickens [29], has a broad range of avian hosts and is considered the predominant avian *Cryptosporidium* species. In China, *C. baileyi* has been reported in a wide variety of birds, including chickens, quails, ostriches, Pekin ducks, domestic pigeons, geese, as well as some pet birds [4, 6, 9, 16, 28, 30]. Evidence has shown that *C. baileyi* causes respiratory disease and production loss in chickens, causing reduced weight gain in broilers and decreased egg production in

layer chickens [31]. One study showed that *C. baileyi* is one cause of Newcastle disease and/or avian influenza vaccination failure in poultry farms [32]. Given the economic importance of *C. baileyi*, sufficient attention should be paid to to this pathogen.

C. meleagridis has been detected in various avian hosts, including chickens, turkeys, cockatiels, pigeons, quails as well as some pet birds [4, 9, 16, 28, 33, 34, 35]. *C. meleagridis* has also been frequently detected in humans worldwide, especially in immunocompromised individuals, such as neonates and HIV/AIDS patients [2]. In China, *C. meleagridis* has been detected in diarrheic children in Wuhan [37], HIV-positive patients in Henan [37] and pediatric patients in Shanghai [38]. *C. meleagridis* is an emerging human pathogen and constitutes the third most common human-pathogenic *Cryptosporidium* species after *C. hominis* and *C. parvum* [39–41]. Moreover, molecular studies revealed that identical *C. meleagridis* subtypes were shared between humans and birds in the same location in Sweden, Peru and China [42], suggesting cross-species transmission of *C. meleagridis* between birds and humans. The detection of *C. meleagridis* detection indicates that chickens may act as a source of infection and a mechanical vector by shedding oocysts into the environment.

Surprisingly, among the 132 test-positive chicken fecal samples, *C. baileyi* was detected in the minority (40%) of samples, while *C. meleagridis* was detected in the majority (60%). The prevalence of *C. meleagridis* in the present study is significantly higher than that of the typical avian species *C. baileyi* [2, 6, 9, 20, 28]. The predominance of *C. meleagridis* among the chicken fecal samples in the present study is consistent with the results of a previous study in poultry in Brazil [43]. Because of the lack of related epidemiological data on cryptosporidiosis in mammals/humans in the investigated areas, the source of infection of domesticated chickens with *C. meleagridis* remains to be elucidated. Whether chickens acquire the infection by contamination of water, feed and/or litter in poultry houses with oocysts from human origin requires further investigation.

To date, at least nine subtype families (IIIa to IIIi) of *C. meleagridis* have been identified by nucleotide sequence analysis of the *gp*60 gene [5, 44, 45]. In the present study, three subtype families of *C. meleagridis*, IIIb, IIIe and IIIg, were detected in chickens. IIIbA21G1R1a, IIIbA24G1R1, IIIbA23G1R1c, IIIeA17G2R1 and IIIeA26G2R1, all of which have previously been reported sporadically in birds [9, 43], but predominantly in humans, especially those with a travel history to Asia [15, 36, 37, 46]. For example, subtype IIIbA23G1R1c, the predominant subtype found here, had previously been isolated from a Swedish patient with a history of travel to Malaysia, while other variants of this subtype, IIIbA23G1R1a and IIIbA23G1R1b, were reported in patients who had traveled to other developing countries (Indonesia or Thailand) prior to infection [15, 46]. Similarly, subtype IIIbA24G1R1, IIIbA21G1R1a and IIIeA17G2R1 infections had previously been linked to travel to Asian countries (China, Thailand or Vietnam). Hence, this information highlights that foreign travel is a significant risk factor for infection with *C. meleagridis.* Moreover, subtype IIIeA26G2R1 identified in chickens in this study was also previously identified in HIV-positive patients in China [37]. This information indicates the cross-transmission of cryptosporidiosis between chickens and humans in this region. Therefore, to better prevent human cryptosporidiosis,

specific management measures are needed on poultry farms, including adhering to an appropriate feeding model, as well as strict hygiene and waste management procedures.

Conclusions

This is the first large-scale molecular study on the occurrence and genetic identity of *Cryptosporidium* in farm-raised chicken in the Guangdong. Province, China. Two species, *C. meleagridis* and *C. baileyi* and were identified. Five of the six subtypes of *C. meleagridis* detected in this study matched those identified in humans. The dominance of *C. meleagridis* infection among chickens and the detection of zoonotic subtypes IIIbA21G1R1a and IIIbA24G1R1 are indicative of cross-transmission of cryptosporidiosis between chickens and humans. Domestic chickens are of public health significance as potential reservoirs of zoonotic *Cryptosporidium*. Further epidemiological investigations are needed to confirm the source of infection of domesticated chickens with *C. meleagridis*.

Abbreviations

SSU rRNA: a portion the small subunit of nuclear ribosomal RNA gene;

*pgp*60**:** a portion of the 60 kDa glycoprotein gene

C. meleagridis: *Cryptosporidium meleagridis*

C. baileyi: Cryptosporidium baileyi

Declarations

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Availability of data and materials

The data supporting the conclusions of this article are included within the article and its additional file. Seven distinct SSU rRNA sequences and subtypes sequences of *C. meleagridis* were deposited under GenBank accession numbers OK560460- OK560466 and OK562693-OK562699, respectively.

Authors' contributions

XJ, MS and MQ planned the study. XL□LX□NQ and ML collected samples. SL, JL and MH undertook the laboratory and analytical work. XL and HC wrote the manuscript, with active inputs from JZ and JH. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Fayer R. Taxonomy and species delimitation in *Cryptosporidium*. Exp Parasitol. 2010;124:90–97.
2. Ryan UM. *Cryptosporidium* in birds, fish and amphibians. Exp Parasitol. 2010;124:113–120.
3. Santín M. Clinical and subclinical infections with *Cryptosporidium* in animals. 2010;NZ Vet J. 61:1–10.
4. Wang R, Jian F, Sun Y, Hu Q, Zhu J, Wang F, et al. Large-scale survey of *Cryptosporidium* spp. in chickens and Pekin ducks (*Anas platyrhynchos*) in Henan, China: prevalence and molecular characterization. Avian Pathol. 2010;39:447–51.
5. Baroudi D, Khelef D, Goucem R, Adjou KT, Adamu H, Zhang H, et al. Common occurrence of zoonotic pathogen *Cryptosporidium meleagridis* in broiler chickens and turkeys in Algeria. Vet Parasitol. 2013;196:334–40.
6. Wang L, Xue X, Li J, Zhou Q, Yu Y, Du A. Cryptosporidiosis in broiler chickens in Zhejiang Province, China: molecular characterization of oocysts detected in fecal samples. Parasite. 2014;21:36.
7. Nakamura AA, Meireles MV. *Cryptosporidium* infections in birds-a review. Rev Bras Parasitol Vet. 2015;24:253–67.
8. Holubová N, Sak B, Horčičková M, Hlásková L, Květoňová D, Menchaca S, et al. *Cryptosporidium avium* n. sp. (Apicomplexa: Cryptosporidiidae) in birds. Parasitol Res. 2016;115:2243–51.

9. Liao Cong, Wang T, Koehler AV, Fan Y, Hu M, Gasser RB. Molecular investigation of *Cryptosporidium* in farmed chickens in Hubei Province, China, identifies 'zoonotic' subtypes of *C. meleagridis*. Parasit Vectors. 2018;11:484.
10. Ryan UM, Xiao L, Read C, Sulaiman IM, Monis P, Lal AA, et al. A redescription of *Cryptosporidium galli* Pavlasek, 1999 (Apicomplexa: Cryptosporidiidae) from birds. J Parasitol. 2003;89:809–13.
11. Zhou L, Kassa H, Tischler ML, Xiao L. Host-adapted *Cryptosporidium* spp. in Canada geese (*Branta canadensis*). Appl Environ Microbiol. 2004;70:4211–5.
12. Ng J, Pavlasek I, Ryan U. Identification of novel *Cryptosporidium* genotypes from avian hosts. Appl Environ Microbiol. 2006; 72:7548–53.
13. Helmy YA, Krücken J, Abdelwhab EM, Samson-Himmelstjerna G, Hafez HM. Molecular diagnosis and characterization of *Cryptosporidium* spp. in turkeys and chickens in Germany reveals evidence for previously undetected parasite species. PLoS One. 2017;12:e0177150.
14. Xiao.Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol. 2010;124:80–9.
15. Stensvold CR, Beser J, Axén C, Lebbad M. High applicability of a novel method for *gp*60-based subtyping of *Cryptosporidium meleagridis*. J Clin Microbiol. 2014;52:2311–9.
16. Li Juan, Lin X, Zhang L, Qi N, Liao S, Lv Mi, et al. Molecular characterization of *Cryptosporidium* spp. in domestic pigeons (*Columba livia domestica*) in Guangdong Province, Southern China. Parasitol Res. 2015;114:2237–41.
17. Xiao L, Singh A, Limor J, Graczyk TK, Gradus S, Lal A. Molecular characterization of *Cryptosporidium* oocysts in samples of raw surface water and wastewater. Appl Environ Microbiol. 2004;67:1097–101.
18. Wang Y, Yang W, Cama V, Wang L, Cabrera L, Ortega Y, et al. Population genetics of *Cryptosporidium meleagridis* in humans and birds: evidence for cross-species transmission. Int J Parasitol. 2014;44:515–21.
19. Gong Z, Kan Z, Huang J, Fang Z, Liu X, Gu Y, et al. Molecular prevalence and characterization of *Cryptosporidium* in domestic free-range poultry in Anhui Province, China. Parasitol Res. 2021;120:3519–27.
20. Santana BN, Kurahara B, Nakamura AA, da Silva Camargo V, Ferrari, ED, et al. Detection and characterization of *Cryptosporidium* species and genotypes in three chicken production systems in Brazil using different molecular diagnosis protocols. Prev Vet Med. 2018;151:73–8.
21. Kassouha M, Soukkarieh C, Alkhaled A. First genotyping of *Cryptosporidium* spp. in pre-weaned calves, broiler chickens and children in Syria by PCR-RFLP analysis. Vet Parasitol. 2016;225:86–90.
22. Hamidinejat H, Jalali MHR, Jafari RA, Nourmohammadi K. Molecular determination and genotyping of *Cryptosporidium* spp. in fecal and respiratory samples of industrial poultry in Iran. Asian Pac J Trop Med. 2014;7:517–20.
23. Soltane R, Guyot K, Dei-Cas E, Ayadi A. Prevalence of *Cryptosporidium* spp. (Eucoccidiorida: Cryptosporiidae) in seven species of farm animals in Tunisia. Parasite. 2007;14:335–8.

24. Hijjawi N, Mukbel R, Yang R, Ryan U. Genetic characterization of *Cryptosporidium* in animal and human isolates from Jordan. Vet Parasitol. 2016;228:116–20.
25. Ewald MPC, Martins FDC, Caldart ET, Vieira FEG, Yamamura MH, Sasse JP, et al. The first study of molecular prevalence and species characterization of *Cryptosporidium* in free-range chicken (*Gallus gallus domesticus*) from Brazil. Rev Bras Parasitol Vet. 2017;26:472–8.
26. Santín M, Trout JM, Xiao L, Zhou L, Greiner E, Fayer R. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet Parasitol. 2004;122:103–17.
27. Amer S, Honma H, Ikarashi M, Tada C, Fukuda Y, Suyama Y, et al. *Cryptosporidium* genotypes and subtypes in dairy calves in Egypt. Vet Parasitol. 2010;169:382–6.
28. Wang R, Wang F, Zhao J, Qi M, Ning C, Zhang L, et al. *Cryptosporidium* spp. in quails (*Coturnix coturnix japonica*) in Henan, China: molecular characterization and public health significance. Vet Parasitol. 2012;187:534–7.
29. Current W, Upton SJ, Haynes TB. The life cycle of *Cryptosporidium baileyi* n. sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. J Protozool. 1986;33:289–96.
30. Zhao W, Zhou H, Ma T, Cao J, Lu G, Shen Y. PCR-Based Detection of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in Farm-Raised and Free-Ranging Geese (*Anser anser f. domestica*) From Hainan Province of China: Natural Infection Rate and the Species or Genotype Distribution. Front Cell Infect Microbiol. 2019;9:416.
31. Goodwin MA, Brown J, Resurreccion RS, Smith JA. Respiratory coccidiosis (*Cryptosporidium baileyi*) among northern Georgia broilers in one company. Avian Dis. 1996;40:572–5
32. Eladl AH, Hamed HR, Khalil MR. Consequence of Cryptosporidiosis on the immune response of vaccinated broiler chickens against Newcastle disease and/or avian influenza. Vet Res Commun. 2014;38:237–47.
33. Laatamna AE, Holubova N, Sak B, Kvac M. *Cryptosporidium meleagridis* and *C. baileyi* (Apicomplexa) in domestic and wild birds in Algeria. Folia Parasitol (Praha). 2017;64:2017.018.
34. Abe N, Iseki M. Identification of *Cryptosporidium* isolates from cockatiels by direct sequencing of the PCR-amplified small subunit ribosomal RNA gene. Parasitol Res. 2004;92:523–6.
35. Qi M, Wang R, Ning C, Li X, Zhang L, Jian F, et al. *Cryptosporidium* spp. in pet birds: genetic diversity and potential public health significance. Exp Parasitol. 2011;128:336–40.
36. Wang T, Fan Yi, Koehler AV, Ma G, Li T, Hu M, et al. First survey of *Cryptosporidium*, *Giardia* and *Enterocytozoon* in diarrhoeic children from Wuhan, China. Infect Genet Evol. 2017;51:127–31.
37. Wang L, Zhang H, Zhao X, Zhang L, Zhang G, Guo M, et al. Zoonotic *Cryptosporidium* species and *Enterocytozoon bieneusi* genotypes in HIV-positive patients on antiretroviral therapy. J Clin Microbiol. 2013;51:557–63.
38. Feng Y, Wang L, Duan L, Gomez-Puerta LA, Zhang L, Zhao X, et al. Extended outbreak of cryptosporidiosis in a pediatric hospital, China. Emerg Infect Dis. 2012;18:312–4.

39. McLauchlin J, Amar C, Pedraza-Díaz S, Nichols GL. Molecular epidemiological analysis of *Cryptosporidium* spp. in the United Kingdom: results of genotyping *Cryptosporidium* spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. J Clin Microbiol. 2000;38:3984–90.
40. Matos O, Alves M, Xiao L, Cama V, Antunes F. *Cryptosporidium felis* and *C. meleagridis* in persons with HIV, Portugal. Emerg Infect Dis. 2004;10:2256–7.
41. Cama VA, Bern C, Roberts J, Cabrera L, Sterling CR, Ortega Y, et al. *Cryptosporidium* species and subtypes and clinical manifestations in children, Peru. Emerg Infect Dis. 2008;14:1567–74.
42. Silverlås C, Mattsson JG, Insulander M, Lebbad M. Zoonotic transmission of *Cryptosporidium meleagridis* on an organic Swedish farm. Int J Parasitol. 2012;42:963–7.
43. da Cunha MJR, Cury MC, Santín M. Molecular characterization of *Cryptosporidium* spp. in poultry from Brazil. Res Vet Sci. 2018;118:331–5.
44. Feng Y, Lal AA, Li N, Xiao L. Subtypes of *Cryptosporidium* spp. in mice and other small mammals. Exp Parasitol. 2011;127:238–42.
45. Abal-Fabeiro JL, Maside X, Llovo J, Bartolomé C. Aetiology and epidemiology of human cryptosporidiosis cases in Galicia (NW Spain), 2000-2008. Epidemiol Infect. 2015;143:3022–35.
46. Lebbad M, Winiecka-Krusnell J, Stensvold CR, Beser J. High Diversity of *Cryptosporidium* Species and Subtypes Identified in Cryptosporidiosis Acquired in Sweden and Abroad. Pathogens. 2021;10:523.

Tables

Table 1
Prevalence and species/subtypes of *Cryptosporidium* spp. in broiler chickens in Guangdong Province, China

Location	Farms	No. sample	No. positive	% (95% CI)	*Cryptosporidium* species		
					No. of positive samples of *C. baileyi* (%)	No. of positive samples of *C. meleagridis* (%)	No. of positive samples of mixed infection (%)
Qingyuan	14	294	28	9.5 (6.1-12.9)	10 (3.4)	18 (6.1)	
Maoming	10	283	40	14.1 (10.1-18.2)	6 (2.1)	31 (11.0)	3 (1.1),
Huizhou	9	227	29	12.8 (8.4-17.2)	23 (10.1)	5 (2.2)	1 (0.4)
Yangjiang	4	106	25	23.6 (15.4-31.8)	4 (3.8)	19 (17.9)	2 (1.9)
Yuedong	4	59	7	11.9 (3.4-20.4)	5 (8.5)	2 (3.4)	
Shanwei	2	32	3	9.4 (0-20.1)		3 (9.4)	
Total		1001	132	13.2 (11.1-15.4)	48 (4.8)	78 (7.8)	6 (0.6),

Table 2
Cryptosporidium spp identified among different age groups of broiler chickens in Guangdong Province, China

Age group	No. sample	No. positive	% (95% CI)	*Cryptosporidium* species		
				No. of positive samples of *C. baileyi* (%)	No. of positive samples of *C. meleagridis* (%)	No. of positive samples of mixed infection (%)
<30d	169	14	8.3 (6.7-9.9)	1 (0.6)	13 (7.7)	
31-60d	440	53	12.0 (11.2-12.8)	14 (3.2)	37 (8.4)	2 (0.5),
61-90d	348	62	17.8 (16.7-18.9)	30 (8.6)	28 (8.0)	4
>90d	44	3	6.8 (2.7-10.9)	3 (6.8)		
Total	1001	132	13.2 (11.1-15.4)	48 (4.8)	78 (7.8)	6 (0.6)

Figures

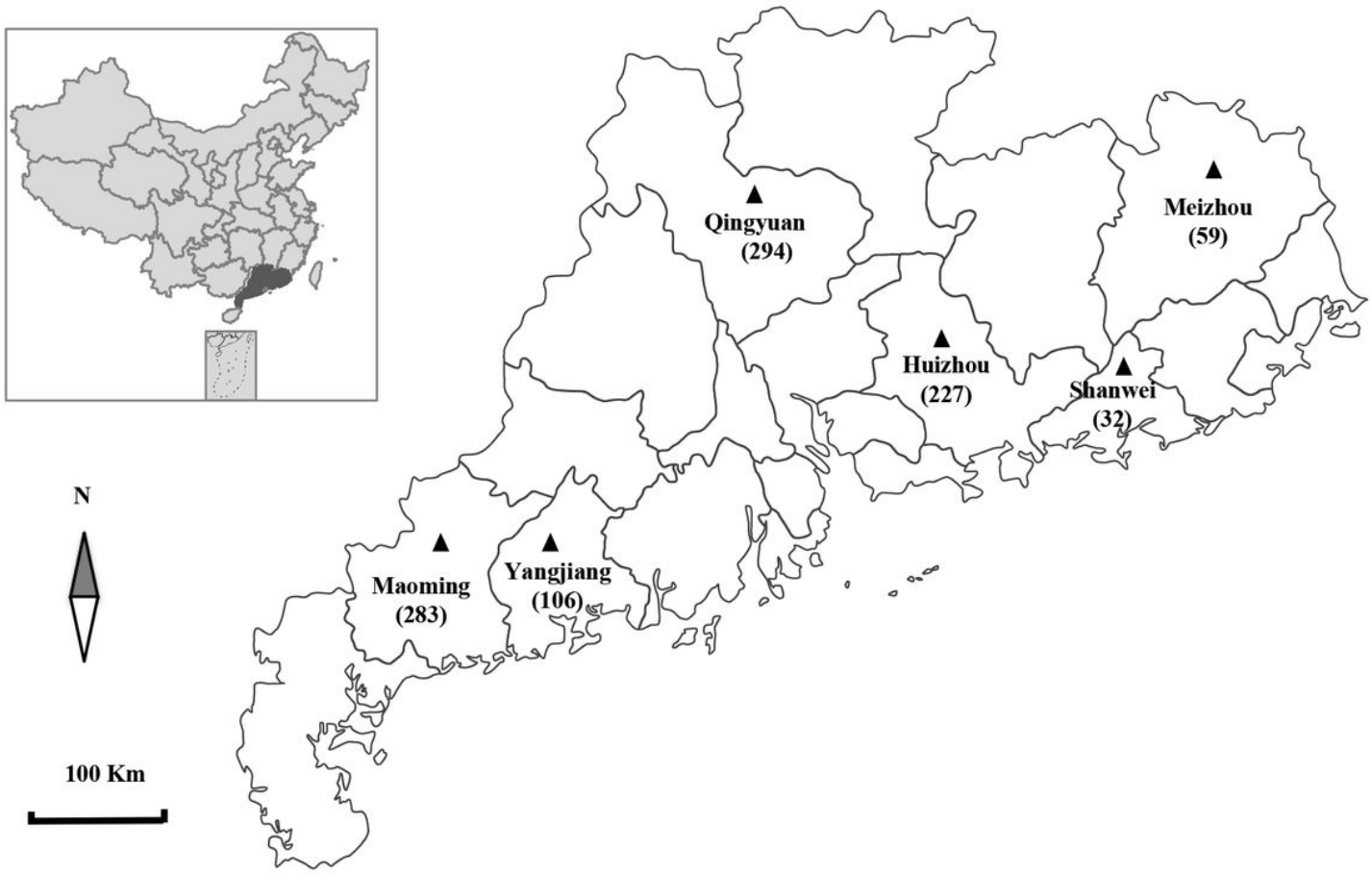

Figure 1

Map of Guangdong showing the locations of the studied cities and number of chicken sample (numbers in parentheses) with geographic distribution. ▲ locations.

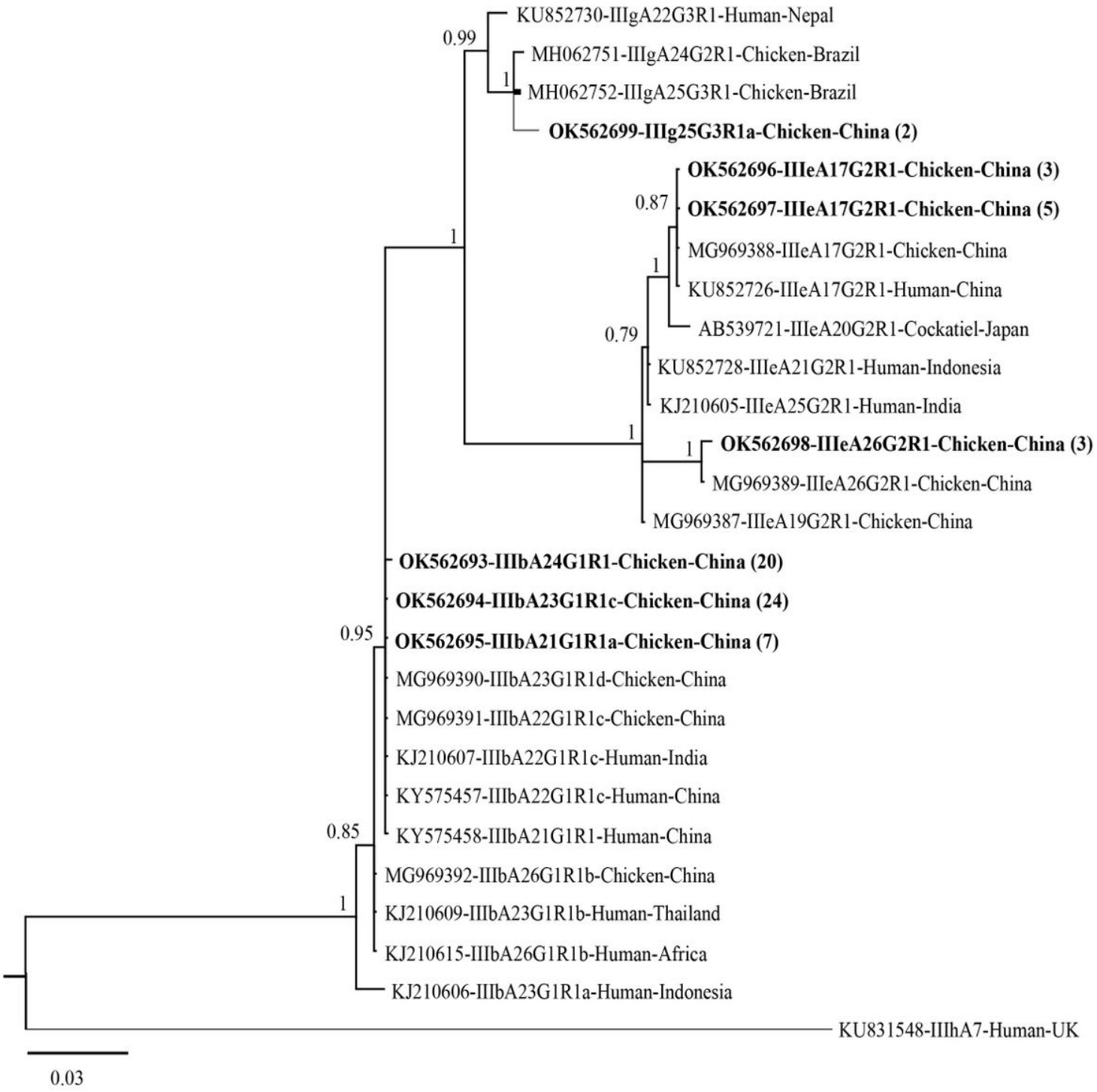

Figure 2

Phylogenetic relationship of the nuclear 60-kDa glycoprotein gene (pgp60) of Cryptosporidium meleagridis in chickens by Bayesian inference (BI). Posterior probabilities of > 0.95 are indicated at all major nodes. Subtypes tested in this study are labeled after the specimen numbers. The scale-bar represents the number of substitutions per site.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- GraphicalAbstract.jpg