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Abstract of Dissertation

Applicant

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Title :

Studies on molecular epidemiology of cryptosporidiosis in poultry and young ruminants

(家禽及び幼若反芻動物におけるクリプトスポリジウム症の分子疫学に関する研究)

Abstract

Cryptosporidium spp. are enteric opportunistic protozoan parasites that infect a wide range of hosts including humans, domestic and wild animals, and more than 30 avian hosts worldwide. It affects the distal small intestine and can affect the respiratory tract in both immunocompetent and immunocompromised individuals, resulting in watery diarrhea. Therefore, cryptosporidiosis is considered to be of significant economic, veterinary, and medical importance.

In chapter 1, this study investigated the prevalence of *Cryptosporidium* spp. in poultry at open live bird markets in Bangladesh. However, there is no information regarding *Cryptosporidium* spp. in poultry in Bangladesh. Accordingly, a total of 197 samples were randomly collected from poultry at open live bird markets in Bangladesh and screened for the detection of *Cryptosporidium*. Subsequent nested PCR targeting the 18S rRNA gene revealed that 15.7% (31/197) of the samples were *Cryptosporidium* positive. Of these 31 samples, 17 were *Cryptosporidium baileyi* (8.7%), 12 were *Cryptosporidium meleagridis* (6.0%), and 2 were *Cryptosporidium parvum* (1.0%). Nucleotide sequence analysis of the GP60 gene of the *C. meleagridis* revealed that two subtypes (IIIbA21G1R1 and IIIbA23G1R1), which were found in the broiler, native and sonali chickens and a pigeon, matched those previously reported in humans and poultry. I identified two novel subtypes (IIIbA21G2R1 and IIIbA20G2R1) in sonali chickens, a broiler chicken, and a layer chicken. I also amplified the GP60 gene of *C. parvum* and found two subtypes (IIaA11G2R1 and IIaA13G2R1) in a sonali and a broiler chicken that were previously reported in the calf. These findings suggest that poultry can be a source of cryptosporidial infections for humans and animals in Bangladesh. This is the first molecular investigation of *Cryptosporidium* genotypes and subtypes in poultry at open live bird markets in Bangladesh.

In chapter 2, this study evaluated to molecularly characterize the *Cryptosporidium* spp. found in calf feces in Japan. A total of 80 pre-weaned beef and dairy calves' diarrhoeic fecal specimens were collected from nine different prefectures in Japan. A nested polymerase chain reaction targeting the small subunit 18S rRNA and GP60 genes were used to detect the *Cryptosporidium* genotypes and subtypes. 83.8% (67 out of 80) of the specimens were positive for *Cryptosporidium* spp.; *Cryptosporidium* was found in both beef and dairy calves. *Cryptosporidium parvum* was the predominant species, detected in 77.5% (31/40) of beef calves and 80% (32/40) of dairy calves. *Cryptosporidium bovis* was also detected, 5.0% (2/40) of dairy calves, and *C. ryanae* was also found 2.5% (1/40) of dairy calves. One mixed-species infection, 2.5% (1/40) was detected in a beef calf having *C. parvum, and C. ryanae*. I detected the most common subtype of *C. parvum* (i.e., IIaA15G2R1), as well as other subtypes (i.e., IIaA14G3R1, IIaA14G2R1, and IIaA13G1R1) that have not previously been detected in calves in Japan. My results demonstrate the wide diversity of *Cryptosporidium* infection in calves in Japan.

In chapter 3, the studies on *Cryptosporidium* infections of animals in Turkey mostly rely on microscopic observation. Few data are available regarding the prevalence of *Cryptosporidium* genotypes and subtypes infection. This study aims to analyze the detection of *Cryptosporidium* genotypes and subtypes in young ruminants. A total of 415 diarrheic fecal specimens from young ruminants were examined for the *Cryptosporidium* detection by the use of nested PCR of the small subunit ribosomal RNA (SSU rRNA) gene and the highly polymorphic 60 kDa glycoprotein (gp60) gene followed by sequence analyses. The results of this study revealed that 25.6% (106 of 415) of the specimens were positive for *Cryptosporidium* spp. infection. I identified 27.4% (91/333), 19.4% (13/67), and 13.4% (2/15) of positivity in calves, lambs and goat kids, respectively. Genotyping of the SSU rRNA indicated that almost all positive specimens were of C. *parvum*, except for one calf which was of *C. bovis*. Sequence analysis of the gp60 gene revealed the most common zoonotic subtypes (IIa and IId) of *C. parvum.* I detected 11 subtypes (IIaA11G2R1, IIaA11G3R1, IIaA12G3R1, IIaA13G2R1, IIaA13G4R1, IIaA14G1R1, IIaA14G3R1, IIaA15G2R1, IIdA16G1, IIdA18G1, and IIdA22G1); three of them (IIaA12G3R1, IIaA11G3R1, and IIaA13G4R1) was novel subtypes found in calves and lambs. Additionally, three subtypes (IIaA11G2R1, IIaA14G3R1, and IIdA16G1) were detected in young ruminants for the first time in Turkey. This results indicate the high infection of *Cryptosporidium* in Turkey and suggest that calves, lambs, and goat kids are likely a major reservoir of *C. parvum* and a potential source of zoonotic transmission.

In conclusion, this study emphasizes the molecular characterization of *Cryptosporidium* parasites. It also figures out the genetic diversity of *Cryptosporidium* genotypes and subtypes family in different hosts and geographically different locations. Here, the zoonotic subtype IIa of *C. parvum* was identified in poultry and young ruminants, and another zoonotic subtype IId of *C. parvum* was identified in calves. People and other animals could be infected with this potential zoonotic pathogen. Therefore, it is important to consider *Cryptosporidium* as a risk for both human and animal health, and the economy. It would need to take precautions, growing awareness, and practice of good hygiene everywhere for the people to prevent this zoonotic infection.