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Case Report

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A case report of *Apatemon gracilis* (Szidat, 1928) infection in domestic geese in mainland China

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Abstract: This is the first report of the diagnosis and treatment of *Apatemon gracilis* infection in domestic geese in mainland China confirmed at the molecular level. The mortality of geese reached 10% (150/1500). Necropsy examinations confirmed hemorrhagic inflammation of the small intestine and blood and catarrhal mucus in the enteric cavity. A large number of flukes were found in the first third of the small intestine, with one end buried in the intestinal mucosa, forming lesions at the absorption sites. The ITS-1 sequence of the parasites was amplified by polymerase chain reaction and sequence analysis confirmed that the isolated worms were Apatemon gracilis (Szidat, 1928). Expulsion of the parasites with praziquantel effectively controlled the disease on the goose farm.

Key words: Goose, *Apatemon gracilis*, internal transcribed spacer-1, polymerase chain reaction, diagnosis

1. Introduction

Geese eat many kinds of aquatic organisms, including intermediate hosts of parasites, which can lead to the development of parasitic disease (1). It has been well documented that domestic geese can be infected with a large number of intestinal helminths (2,3). Although helminthic infections usually have a symptomless manifestation in goose flocks, these worms may have an additive effect on production performance, even mortality, in combination with other factors, such as malnutrition and crowding (4).

Apatemon gracilis is an intestinal trematode that has been frequently reported in ducks in certain geographic areas of Europe (5,6). It also has been found in the intestine of birds (7,8) and ducks are its main hosts (9). This study describes a case of *A. gracilis* infection in a domestic goose flock. To the best of our knowledge, this is the first report of the occurrence of this parasitic disease in mainland China confirmed at the molecular level. Results from this study should prove useful for clinical diagnosis and treatment of *A. gracilis* infection in other areas.

2. Case history

The goose flock was located in Zhenjiang, Jiangsu Province, China, comprising 1500 birds of 84 days old. Birds were fed with rice and green forage. A tent was set up on a river bank for use as a bird house. Three weeks prior to clinical diagnosis, the geese started to show symptoms of decreased appetite, slow growth, paralysis, and diarrhea producing whitish and reddish feces. By the time that the birds were delivered to the clinic at the animal hospital of Yangzhou University, 450 birds still had clinical symptoms and 150 birds had died. The surviving birds were administered norfloxacin and gentamicin, but neither antibiotic was effective. According to the owner of the farm, the same disease occurs from September to October every year. Other goose farms in the same area reported similar problems.

Necropsy conducted on five dead geese showed that the bodies were severely emaciated with hemorrhagic enteritis. A large number of whitish worms the size of sesame seeds were found in the first third of the small intestine (Figure 1). One end of these parasites was buried in the intestinal mucosa, which formed lesions at the adsorption sites. The parasites were easily scrapped off by gently pressing scissors against the tissue, and they were collected for morphological observation. No remarkable pathogenic changes were observed in other organs.

Autopsied brain tissues were placed in a saline solution (1:5; w/v) and ground into a fine powder by repeated freeze/thaw cycles. After centrifugation at 2500 × *g* for 15 min, the supernatant was collected, then transferred to a penicillin-

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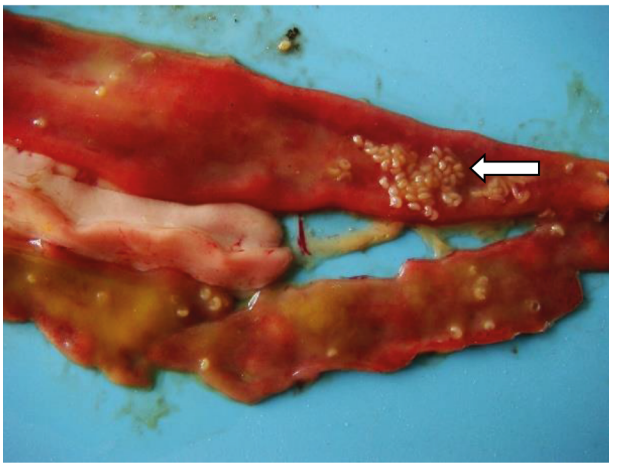

Figure 1. Catarrhal and hemorrhagic inflammation of an autopsied sample of the small intestine. A large number of whitish worms (white arrow) were attached to the intestinal mucosa. Some parasites were removed by excochleation.

streptomycin solution (2000 IU/mL each) and incubated at 37 °C for 1 h. After filtering through a 0.22-μm syringe-driven filter, allantoic cavity inoculation was performed on five 9-day-old specific pathogen-free chicken embryos. For further testing, autopsied tissues of the liver, heart, and spleen were inoculated onto general nutrient agar plates, sheep blood plates, and MacConkey agar plates, as well as nutrient broth. At the same time, blood from diseased geese was used to prepare blood smears to do microscopy by using Wright staining, and the small intestine mucosa and its contents were also examined under a microscope.

To further confirm the identity of the parasites at the molecular level, a pair of primers was designed against the sequences of ITS-1 from a species of the *Apatemon* genus (10). The primer sequences were P1: 5′-CAAGTCCCTATCTGAAACTG-3′ and P2: 5′-CATCGACACACGAGCCGA-3′. Each PCR reaction volume of 25 μL contained 9.5 μL of ddH_2O, 12.5 μL of 2X Easy Taq PCR SuperMix (TransGen Biotech, Beijing, China), 1.0 μL of 10 μM primer (each), and 1.0 μL of genomic DNA (100 ng/μL), which was extracted from the parasites using a DNA extraction reagent (Bao Biotech, Daliang, China). The PCR amplification reaction was performed using a program of predenaturation at 94 °C for 2 min followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 58 °C for 35 s, and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 5 min. The PCR product was analyzed by 1.5% agarose gel and was submitted for sequencing analysis to Sangon Biotech (Shanghai, China).

3. Results and discussion

A total of 1502 worms were collected from five diseased geese. The parasites shared the same morphological properties with an average length of 2.15 mm (n = 10). The cylindrical body was divided into front and rear parts (Figure 2). The front part was 1/3 of the total body length, cup-shaped, and contained an oral sucker and a ventral sucker. The rear part of the body was twice the length of the front part and was cylinder-shaped, containing the reproductive organs. In the experimental detection, all of the five 9-day-old specific pathogen-free chicken embryos, which were inoculated by autopsied brain tissues in the allantoic cavity, survived after incubation for 96 h at 37 °C. Hemagglutination test results of the collected allantoic fluid were consistently negative. Subsequent tests on two generations of further passages all produced negative results. These results excluded virus infection of the geese. After incubation at 37 °C for 24 h, there was no colony formation on general nutrient agar plates, sheep blood plates, or MacConkey agar plates. According to microscopy, there were no susceptive protozoans in blood smears, and there were several trematode eggs bearing an operculum on one end in the small intestine mucosa and its contents had no coccidial oocysts or eggs of other worms observed. Based on these morphological properties and the habitat, the worms were preliminarily identified as the genus *Apatemon* in the family Strigeidae, order Paragonimidae, and class Trematoda. As a result of molecular analysis, a DNA band of 630 bp (Figure 3) was detected by PCR. Its sequences (Figure 4; GenBank Accession No. KY285077) were 100% identical to the ITS-1 sequences of *Apatemon gracilis* found in Great Britain (GenBank accession no. AJ314760.1). These results confirmed that the worms infecting the geese in this case were *Apatemon gracilis* (Szidat, 1928).

Praziquantel (200 mg tablets) was purchased from Weida (Hunan, China). It was given orally to treat the

Figure 2. A parasite isolated from the small intestine of a goose. The worm is divided into front and rear parts (400×).

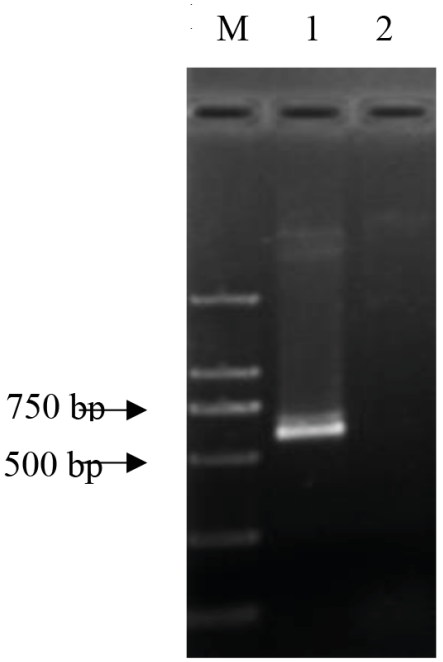

Figure 3. The ITS-1 gene fragment amplified from the genomic DNA of worms isolated from infected geese. M: DL2000 DNA Marker; 1: DNA band amplified from genomic DNA of the parasites; 2: negative control without the parasite genomic DNA template in the PCR reaction.

geese, including both healthy and sick birds, at a dose of 20 mg/kg body weight, once per day for two consecutive days. During the treatment period, geese were given fresh green feed and grain, and water was supplemented with a multiplex vitamin. The bird houses were cleaned daily and all feces were completely removed. At 2–4 days after treatment, a large number of whitish parasites were excreted in the feces and the birds recovered their appetites. A follow-up at 15 days after treatment at the goose farm showed that all birds had regained normal and healthy activities. Five of the isolated sick birds, which were isolated when the disease occured, were picked randomly for fecal analysis and autopsies, and no eggs and parasites were detected.

In the clinic, preliminary diagnosis and identification of helminthic infections in geese are normally based on the habitat sites and the morphological properties of the parasites, which can be observed with the naked eye or under a microscope (1,4). However, trematodes of different genera and species (e.g., the family *Strigeidae*) sometimes look very similar morphologically, which can subsequently lead to misdiagnosis and create a challenge for disease prevention and control (10). Molecular biological methods have been widely used to investigate phylogenetic relationships in parasites to provide comparable quantitative evolutionary information that is not based on morphology or biogeography, and not affected by environmental factors or the subjective judgment of researchers (11). In this study, for the first time, the incidence of *Apatemon gracilis* in mainland China was confirmed based on the ITS-1 sequences at the molecular level.

The life cycle of this fluke requires two intermediate hosts: the first is a freshwater snail and the second includes frogs and freshwater fish in addition to snails. A final host is infected by feeding on the second intermediate host containing metacercariae. These parasites develop into adults in 20 days inside the bodies of aquatic birds (12). The

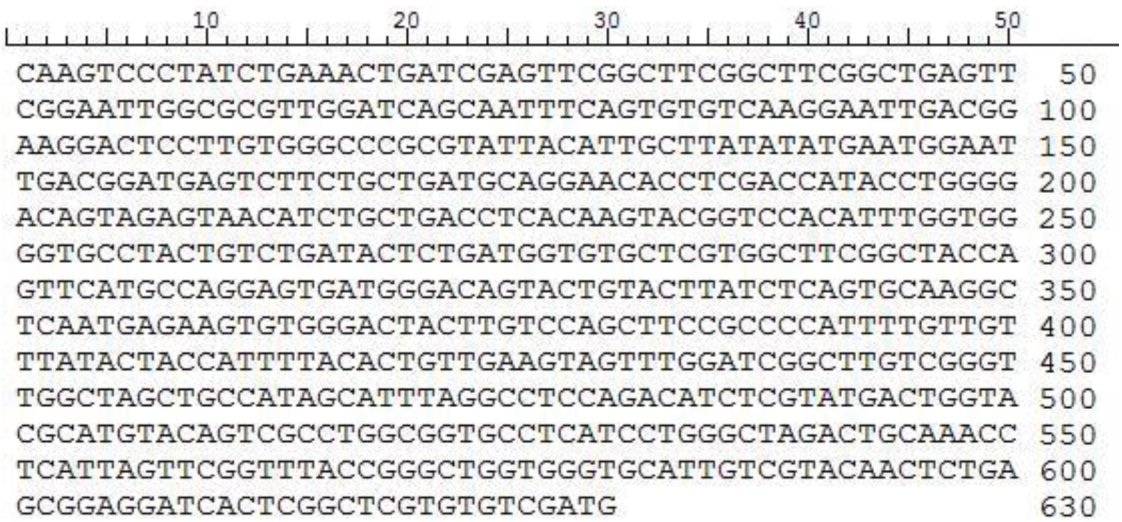

Figure 4. ITS-1 gene sequences of the isolated worms from the infected geese.

cup-shaped front part of this fluke was attached by suckers to the intestinal mucosa. The vessels in the mucosa become congested and then rupture. Afterward, the parasites feed on the blood and the degraded mucosa is digested by secretions from the parasites. Finally, these activities result in hemorrhagic inflammation of the intestinal mucosa (4). The clinical symptoms of heavily infected birds include anemia, hemorrhagic necrotizing enteritis, and even death. This parasitic disease can be preliminarily diagnosed based on the characteristics of lesions. However, attention must be exercised to exclude other parasitic diseases with similar pathogenic changes, such as echinostomiasis and intestinal coccidiosis (13). Echinostomiasis can be diagnosed by a special characteristic of morphology, with thorns at the top of parasites, while in intestinal coccidiosis parasites cannot be seen directly by eye.

Grazing geese are very likely to feed on intermediate hosts, which usually cause some helminthic infections. Upon diagnosis, pastoral farming of birds should be stopped, and broad-spectrum drugs should be used to effectively expel multiple species of worms, including nematodes, trematodes, and tapeworms. Preventive measures should also be taken in the endemic area, which includes eliminating freshwater snails, maintaining hygienic bird houses, heat fermentation of feces, and removal of eggs and worms (4).

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