Atoxoplasma infection in a common mynah (*Acridotheres tristis*)

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Abstract

A carcass of common mynah (*Acridotheres tristis*) from Bangkok was submitted to National Institute of Animal Health (NIAH) for disease diagnosis. At necropsy, enlargement with multifocal necrosis of liver was found. Histopathologically, a lot of intracytoplasmic inclusion bodies were found in hepatic cells, Kupffer cells and sinusoids. By transmission electron microscopy, they were diagnosed as intracytoplasmic merozoites of *Atoxoplasma* spp. and this was the first report case of *Atoxoplasma* infection of bird in Thailand.

Keywords: *Atoxoplasma*, *Acridotheres tristis*, histopathologically, electron microscopy

Introduction

Atoxoplasmosis is a parasitic disease commonly found in passerine birds and wild birds such as common mynah, starling, hill mynah, oriole, canary, sparrow and tanagers (Adkesson *et al*.,2003). In the United States, surveys of the parasite have been done periodically especially in pet bird colonies. *Atoxoplasma* spp. belongs to Apicomplexa group, genus *Atoxoplasma* that is host specific and causes disease in both natural and pet birds (Aiello, 1998). There are two forms of *Atoxoplasma*, intestinal form and circulating form. Intestinal form of *Atoxoplasma* is multiplied by both sexual and asexual reproduction while circulating form is multiplied by only asexual reproduction (McNamee *et al*., 1995; Quiroga *et al*., 2000). Since the intestinal form of *Atoxoplasma* looks like *Isospora* , it is so called extraintestinal coccidian protozoan or tissue invading form of coccidian protozoa (Cooper *et al*., 1989; Levine, 1982; McNamee *et al*., 1995). The hosts are infected by ingestion of sporulated oocysts in feces of infected birds. After ingestion, the infective sporozoites penetrate throught intestinal mucosa, develope schizogony, merogony, gametogony and form unsporulated oocysts. For circulating form, the sporozoites infect mononuclear cells and macrophages and develop merogony in these cells. The infected birds show depression, loss of appetite, diarrhea, ruffled feather, ataxia and muscular tremor. High mortality, a reach to 80% is common in young birds (Aiello, 1998). Clinical signs of respiratory system are occasionally seen in some birds (Charles, 1998). Infected mature birds can shed unsporulated oocysts in feces without any clinical signs. The oocysts sporulate in the environment and are then infective. It is rather difficult to eradicate the parasite since the unsporulated oocysts are resistant to many disinfectants and can survive in the environment for a long time (Aiello, 1998). Lesions caused by the parasite includes enlargement of spleen and liver with multifocal necrosis (McNamee *et al*., 1995). Although the parasite is morphologically similar to coccidian protozoa, but it is resistant to anticoccidial drugs (Aiello, 1998). However, other drugs such as sulfachlorpyrazine and sulfachlorpyridazine can decrease shedding of oocyst from the infected birds. (Norton *et al*., 1995). Detection of this parasite can be done by identifying the parasite in lymphoid cells and macrophages from thin blood film and fecal oocysts by floatation methods. Morphology of the oocyst looks like those of coccidian and it is rather confusing to determine this parasitic oocyst in feces (Speer and Duszynski, 1975). By histopathology, the parasite can be detected in monocytes of several visceral organs including livers, spleens, and lungs (Charles, 1998; Quiroga *et al*., 2000).

This paper was the first report of *atoxoplasma* infection in a naturally infected common mynah (*Acridotheres tristis*) which confirmed by histopathology and transmission electron microscopy.

Materials and methods

Case study:

A carcass of found dead common mynah (*Acricotheres Tristis*) from Bangkok metropolitan was submitted for diagnosis at National Institute of Animal Health, Bangkok.

Pathological examination

At necropsy, parts of heart, lung, liver, spleen, kidney, brain and all parts of gastrointestinal tract were collected and fixed in 10% buffer formalin and processed for histopathological examination. Giemsa staining (Luna, 1968), Pinkerton's staining (Simmon and Gentzknow, 1994) and immunohistochemistry were done to detect *Chlamydia psittaci.*

Microbiological examination

Ground tissues of lung, liver, spleen, kidney and brain were prepared to be 10% suspension in phosphate buffer saline. After centrifugation the supernatant was filtered through 0.45 and 0.2 micron filters and then injected into allantoic sac of 9-11 day embryonic eggs. Virus isolation was blindly passaged. The avian influenza virus in dead embryos was tested by hemagglutination (HA) and hemagglutination inhibition (HI) tests. For bacteriological examination, the specimens were cultured on blood agar and MacConkey agar and identified according to Quinn *et al.*, 1998.

Electron microscopic examination

The formalin fixed tissues were processed for electron microscopic examination , the fixed tissues were cut at 1 cubic millimeter, fixed with 1% osmium tetroxide at 4 °C for 2 hours and then dehydrated with 50%, 70%, 80%, 90% and 100% ethanol alcohol, respectively. The tissues were infiltrated with a combination of absolute alcohol and propylene oxide (ratio 1:1), propylene oxide, a combination of propylene and epoxy resin (ratio 1:1) and epoxy resin, respectively. The processed tissues were embedded in epoxy at 60 °C for 36 hours, cut at 500 °A and taken up on grids. After staining with 5% uranyl acetate and lead citrate, the specimens were studied under transmission electron microscope, JEOL JEM-1200EX.

Results

Pathological finding

At necropsy, enlargement of liver with disseminated pinpoint white necrotic foci was found in emaciated carcass. Histopathologically, increased number of Kuppfer cells, vacuolar degeneration and coagulative necrosis of hepatic cells were observed. In addition, numerous ellipsoidal, pale basophilic

intracytoplasmic inclusion bodies were found in hepatic cells, Kuppfer cells and also freely presented in sinusoids (Fig.2). The inclusion bodies were blue and pale pink when stained with Geimsa and Pinkerton's staining, respectively. By immunohistochemistry, the tissues were negative for *C. psittaci.*

Electron microscopic examination

By transmission electron microscopy, intracytoplasmic merozoites of the parasites were found in Kuppfer cells, hepatic cells and freely distributed in hepatic sinusoids. Clusters of merozoites were surrounding hepatic nuclei (Fig.3) and occasionally in the host cell membrane (Fig.4). The merozoite was ellipsoidal, 4 microns in length and 2 microns in width, with double plasmamembrane (Fig.5). Anterior part of the merozoites was rather sharp in shape with conoid while posterior part was curve. The parasite nucleus was just posterior to the middle of the protozoa and a dense nucleolus was at the periphery. The conoid and other parasitic organelles such as micronemes and spherical bodies were obviously seen. (Fig.6).

Microbiological examination

There was no virus and bacteria isolated from the specimens.

Discussion

In this study, the mynah was naturally infected with *Atoxoplasma* spp. and finally dead since heavy infection of *Atoxoplasma* spp. was detected in liver. Identification of the parasite was done by histopathology and electron microscopy in addition with avian influenza virus H5N1 and bacterial isolation were negative result. The morphological characters of the parasite in this study are corresponded with McNamee *et al.*, (1995). It is important to differentiate *Atoxoplasma* spp. from *Chlamydia psittaci* as *Chlamydia* spp. which is commonly found in birds and some lesions are alike (Anderson *et al.*, 1997). However, their inclusion bodies are different in size. As *Atoxoplasma* spp. ranges 3-5 microns while *C. psittaci* ranges 0.2-0.4 microns. From immunohistochemistry result, *Chlamydia psittaci* was negative.

Detection of *Atoxoplasma* spp. from blood smear and other method like oocyst examination in feces is a low sensitive method since oocyst shedding is rare in clinical cases but detectable in subclinical cases (Cooper *et al.*, 1989; Partington *et al.*, 1989). It is rather difficult to differentiate oocyst of *Atoxoplasma* spp. from that of *Isospora* spp. because both sporulated oocysts have the same number of 2 sporocysts and 4 sporozoites. (McNamee *et al.*, 1995). Therefore, differentiation of oocyst of *Atoxoplasma* spp. from that of *Isospora* spp. by light microscopy alone is not definitive (Speer and Duszynski, 1975). Furthermore, *Atoxoplasma* spp. is occasionally so called "extraintestinal stage of coccidian" (Box, 1975).

In this study, *Atoxoplasma* spp. was diagnosed by transmission electron microscopy that was sensitive for diagnosis of *Atoxoplasma* spp. However, these methods have limitation in that they can be performed only on dead birds. Hence, more sensitive method like polymerase chain reaction (PCR) should be performed, especially in subclinical cases that also can be done in live infected birds (Schrenzel *et al.*, 2001).

It is noticeable that *Atoxoplasma* infection in birds, reported from several places mostly have the same target cells, lymphoid cells and macrophages (McNamee *et al.*, 1995). However, in this study, the parasite was detected mainly in hepatic cells, which corresponded with Kubo *et al.* (2005). Moreover, this report confirmed the presence of *Atoxoplasma* infection of bird in Thailand. Since the parasite is rather resistant to disinfectants and also can survive in the environment for a long time, and transmission of this disease is by oral-fecal route, sanitation in bird colonies especially on pet bird farming should be emphasized. In addition, the infected mature birds can shed infective oocysts without clinical signs, therefore multi-age rearing of pet birds in the same house should be avoided.

Acknowledgments

We thank Dr. Masanori Kubo, National Institute of Animal Health, Japan, Associate Professor Dr. Thaweesak Songserm, Faculty of Veterinary Medicine, Kasetsart University for the useful consultation, Dr. Jira Kongklong, Dr. Tuangtong Patchimasiri and all technicians of Pathology Section, Bacteriology Section and Virology Section, National Institute of Animal Health, Thailand for their kind assistance.

Figure 1 a common mynah (Acridotheres tristis)

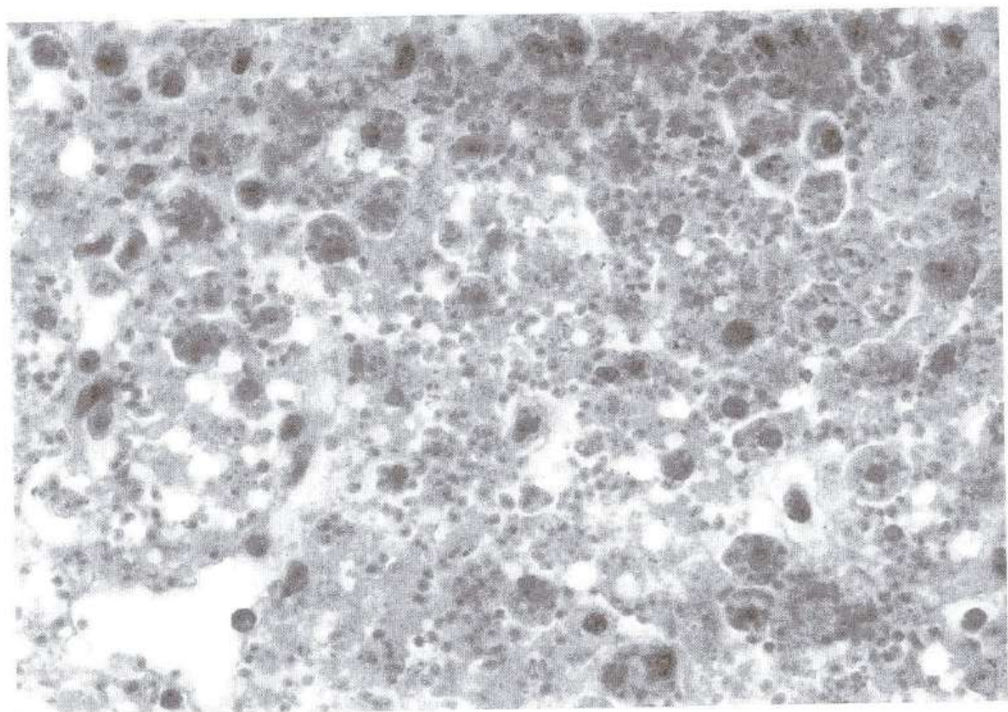

Figure 2 Intracytoplasmic inclusion bodies in liver of a common mynah. It was mainly found in hepatic cells. H&E staining (x 1000)

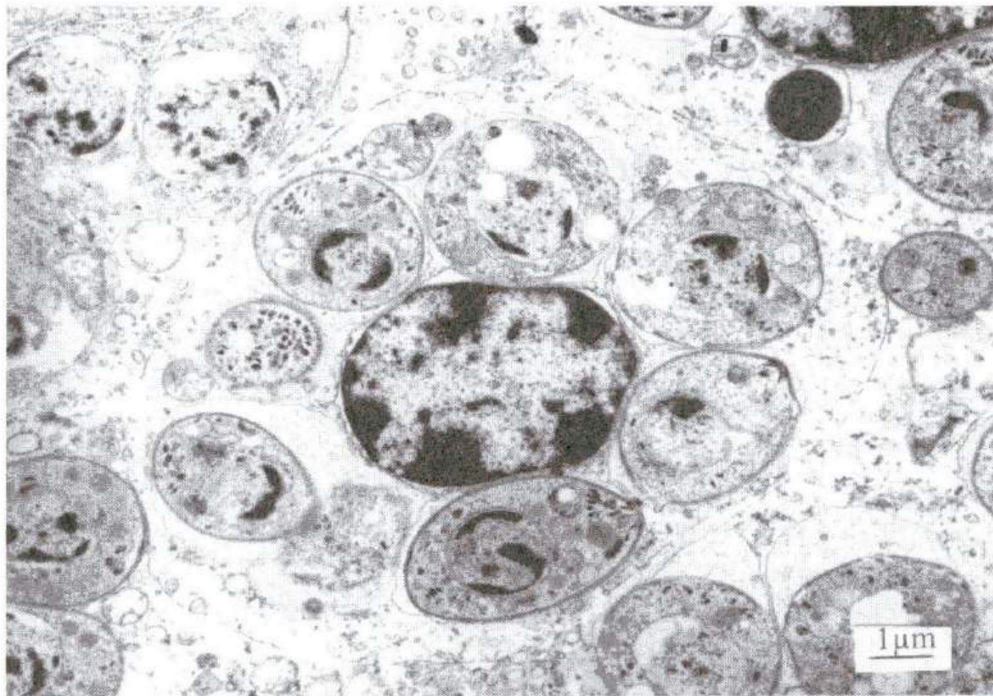

Figure 3 Electronmicrography of Atoxoplasma .Clusters of merozoites were surrounding nucleus of hepatic cell.

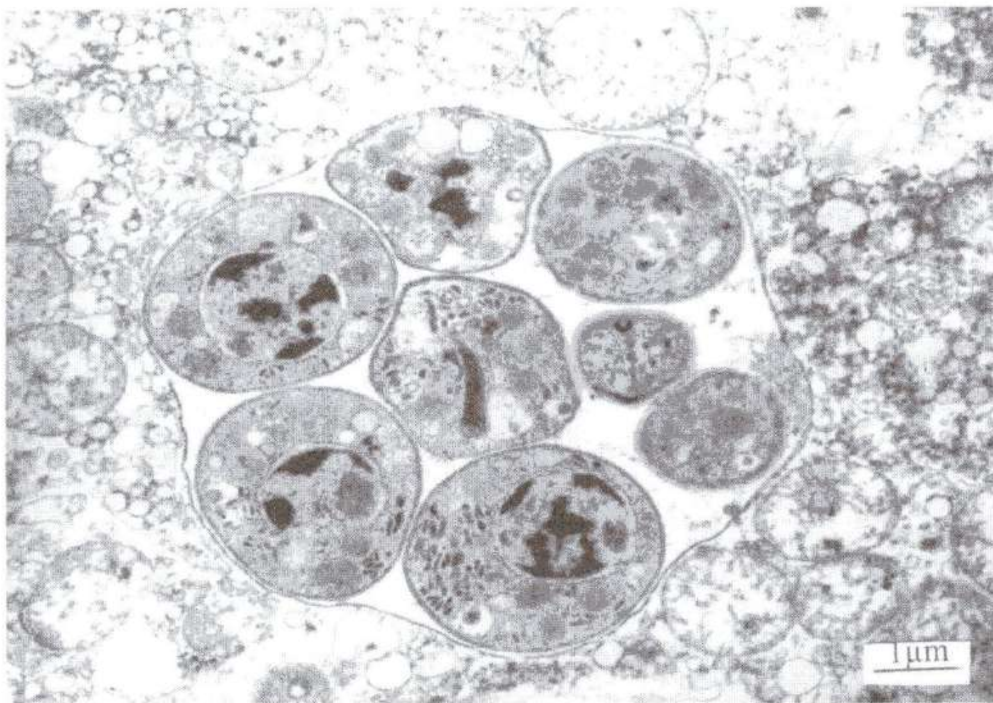

Figure 4 Group of merozoites of Atoxoplasma were found in the host cell membrane.

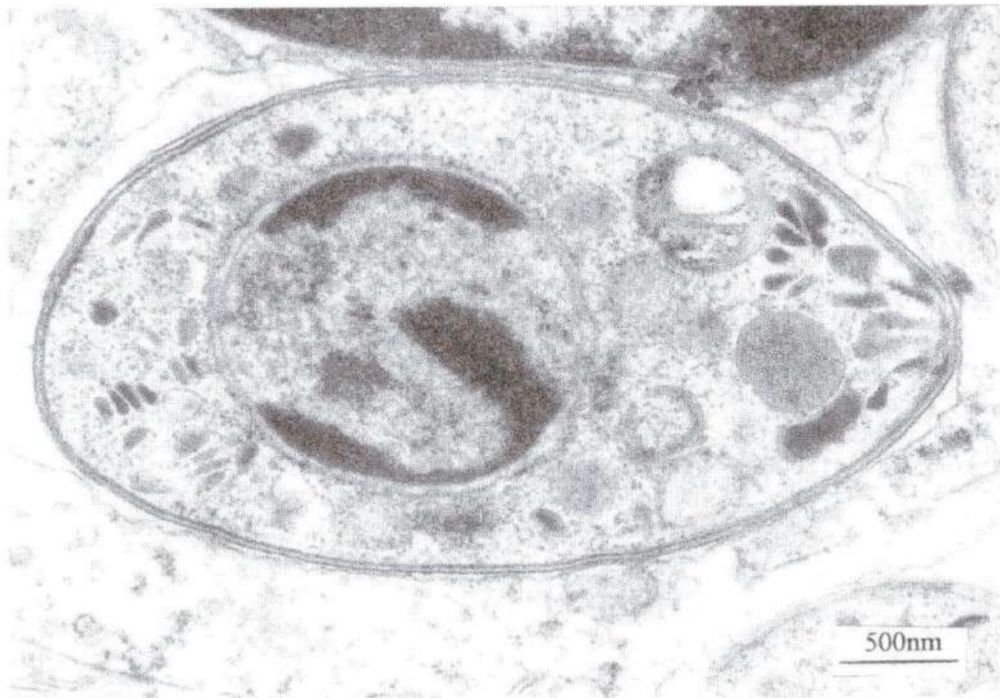

Figure 5 Longitudinal section through a merozoite. The plasmamembrane had 2 layers. The anterior region of the parasite was more point than the posterior end, and the nucleus was just posterior to the middle of the protozoa. A dense nucleolus was visible at the periphery of the nucleus.

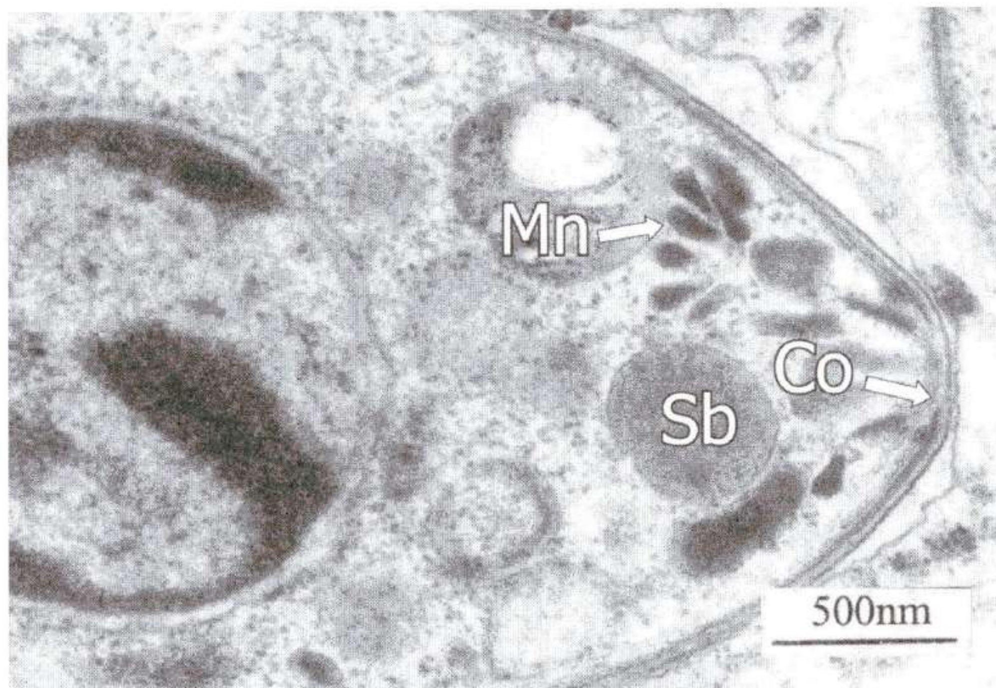

Figure 6 Details of anterior end of merozoite showing conoid(Co),spherical bodies(Sb) and micronemes(Mn).

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Atoxoplasma infection ในนกเอี้ยงสาริกา (*Acridotheres tristis*)

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บทคัดย่อ

นกเอี้ยงสาริกา (*Acridotheres tristis*) ที่ตกลงมาตายอย่างไม่ทราบสาเหตุในกรุงเทพมหานคร ถูกส่งมาชันสูตรโรคที่สถาบันสุขภาพสัตว์แห่งชาติ ผ่าซากพบตับมีขนาดขยายใหญ่ และมีจุดเนื้อตายขนาดเล็กกระจายทั่วไป ผลการตรวจทางจุลพยาธิวิทยาพบ intracytoplasmic inclusion bodies จำนวนมากในเซลล์ตับ Kupffer cells และ sinusoids เมื่อทำการศึกษาด้วยกล้องจุลทรรศน์อิเล็กตรอน พบว่าเป็น intracytoplasmic merozoites ของเชื้อ *Atoxoplasma* spp. รายงานนี้เป็นรายงานการติดเชื้อ *Atoxoplasma* spp. ในนกครั้งแรกในประเทศไทย

คำสำคัญ: *Atoxoplasma* Infection, *Acridotheres tristis*, จุลพยาธิวิทยา, กล้องจุลทรรศน์อิเล็กตรอน