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Detection of *Toxoplasma gondii* in shellfish and fish in parts of China

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ABSTRACT

In this study, a total of 3432 aquatic animals covering eight species were tested by specific polymerase chain reaction (PCR) assays to investigate the presence of *Toxoplasma gondii*, in China. The results showed that, out of 618 *Procambarus clarkii* samples collected from the different areas in southeast China, four samples from Jiangxi province were positive. Of 456 *Hypophthalmichthys molitrix* samples, one sample from Jiangsu province was positive. In addition, there was only one positive sample collected from Shandong province out of 813 *Macrobranchium nipponense* samples. All other samples, including 309 *Cyprinus carpio*, 398 *Concha Ostreae*, 98 *Momopterus albus*, 426 *Penaeus monodon* Fabricius and 309 *Carassius auratus* were negative. The results suggested that the level of *T. gondii* in aquatic animals was low in China. However, the importance of *T. gondii* in aquatic animals should not be ignored. Consumption of contaminated raw shellfish may represent a considerable health threat.

Toxoplasma gondii infects virtually all warm-blooded animals including humans, livestock and marine mammals (Dubey et al., 2008). In humans, the infection is usually either asymptomatic or the cause of mild flu-like symptoms. However, toxoplasmosis can be life threatening in immunocompromised individuals. Moreover, if acquired during pregnancy, toxoplasmosis can cause miscarriage or congenital malformations affecting the brain, eyes or other organs of the foetus. This parasite is also responsible for major economic losses in most classes of livestock through abortions, foetal mummification, stillbirth and neonatal losses, especially in pigs and sheep (Edwards and Dubey, 2013; Kim et al., 2009).

The cat is the final host of *T. gondii*. The oocyst of *T. gondii* is shed in the faeces of infected cats. Soil contamination by *T. gondii* oocysts has been reported from areas where domestic cats are endemic (Dabritz et al., 2007; Ruiz and Frenkel, 1980). In the environment, the oocysts can survive over 1 year under natural conditions (Dumetre and Darde, 2003). Thus, the oocysts can contaminate freshwater following rainfall and enter the nearshore marine environment through freshwater runoff (Fayer et al., 2004; Miller et al., 2002). Humans and domesticated and wild animals become infected by ingesting meat containing encysted bradyzoites, or water and fresh vegetables containing oocysts of *T. gondii* (Benenson et al., 1982; Dubey, 2008).

T. gondii has been found to infect several marine mammal species worldwide, such as seals, blowfish, dolphins, porpoises, whales, sea lions and sea otters (Cabezon et al., 2011; Di Guardo et al., 2011; Dubey et al., 2003; Fayer et al., 2004; Miller et al., 2008). Under natural conditions, *T. gondii* was recently detected in wild shellfish such as *Mytilus californianus* in California (Miller et al., 2008) and in *Mytella guyanensis* and *Crassostrea rhizophorae* in Brazil (Esmerini et al., 2010). The presence of *T. gondii* DNA in Pacific cupped oysters (*Crassostrea gigas*) and true clams (*Tapes decussatus*)

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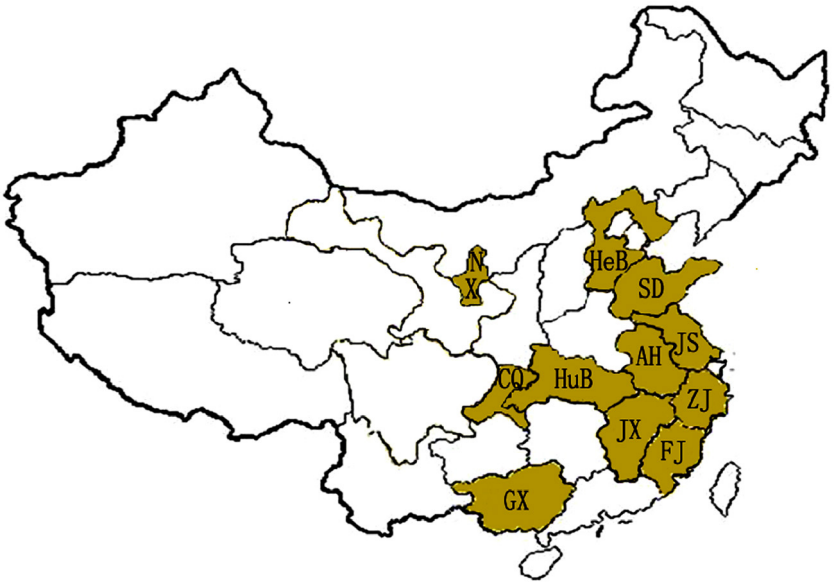

Fig. 1. Sample locations. Shadowed areas were provinces/municipalities in Mainland China surveyed for *T. gondii*. HeB: Hebei province; SD: Shandong province; JS: Jiangsu province; ZJ: Zhejiang province; FJ: Fujian province; AH: Anhui province; HuB: Hubei province; JX: Jiangxi province; CQ: Chongqing municipality; GX: Guangxi Zhuang Autonomous Region; NX: Ningxia Hui Autonomous Region.

was reported in Italy and represented the first observation in Europe (Putignani et al., 2011). The presence of *Toxoplasma* in wild and edible farmed shellfish indicates that consumption of contaminated raw shellfish may represent a considerable health threat.

Aquaculture is a very large industry in China. Until now, data on *T. gondii* prevalence in shellfish or fish from China were missing. In this study, the presence of *T. gondii* in eight kinds of cultured shellfish and fish was investigated in parts of China by polymerase chain reaction (PCR) assays.

Materials and methods

Development of PCR method

Primer design

T. gondii-specific primers were designed based on the *T. gondii*-conserved gene internal transcribed spacer 1 (ITS-1) sequence (GenBank TM accession number AY259044.1) by Primer Premier 5 software. The sense and antisense primers are '5′-TGAATCCCAAGCAAAACAT-3′' and '5′-GGAAGCAATCTGAAAGCAC-3′', respectively. The product of the primers was predicted to be a 341-bp fragment. The primers were synthetised by Shanghai Yingjun Biotechnological Co. Ltd (Beijing, China).

Specificity

A panel of genomic DNA samples from oyster, chicken, crayfish, fish, *Escherichia coli*, *Eimeria tenella*, hypodermal haematopoietic necrosis baculovirus (HHNBV) as well as *T. gondii* (RH strain) were tested for primer specificity. In total, approximately 150 ng of each DNA sample was tested as a template. All of the DNA samples were supplied by the Laboratory of Molecular and Immunological Parasitology, Nanjing Agricultural University, PR China.

Sensitivity

To estimate the sensitivity of the PCR assay, amplification of 10-fold dilutions of *T. gondii* DNA of the RH strain was performed. Dilution series containing from 50 ng to 50 fg of *T. gondii* DNA were tested.

Samples and sample locations

Samples including 309 *Carassius auratus*, 398 *Concha Ostreae*, 426 *Penaeus monodon* Fabricius, 618 *Procambarus clarkii*, 98 *Momopterus albus*, 456 *Hypophthalmichthys molitrix*, 309 *Cyprinus carpio* and 813 *Macrobranchium nipponense* were collected from January 2010 to March 2012. All of the samples were purchased from markets. The origins of the samples covered 11 provinces/municipalities of China, including Eastern China (Shandong Province, Jiangsu Province, Zhejiang Province and Fujian Province), North China (HeBei Province), Central China (Ningxia Hui Autonomous Region, Hubei Province, Anhui Province and Jiangxi Province) and Southwest China (Guangxi Zhuang Autonomous Region and Chongqing Municipality). The locations of the samples are shown in Fig. 1.

Sample DNA extraction

Of the digestive tract tissues, 1 g per sample was collected. These tissues were ground in liquid nitrogen to produce fine powders. The powders were incubated at 55 °C for 8–12 h in lysis buffer (10 mM Tris–HCl, pH 8.0; 100 mM NaCl; 25 mM ethylenediaminetetraacetic acid (EDTA); 1% sodium dodecyl sulphate (SDS); 400 mg mL^{-1} proteinase K). DNA was extracted via phenol, phenol–chloroform and chloroform steps and then precipitated with two volumes