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学　位　論　文　要　旨

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[論文題名]

Evaluation of real-time PCR assay for the detection of *Ascaris suum* contamination in meat and organ meats.

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[要　旨]

Introduction

Ascaris suum known as an intestinal roundworm in swine is distributed worldwide. The parasite infects accidentally to other animals including chickens, cattle and humans. When those accidental hosts are infected, the larvae hatch in the small intestine and migrate through the liver to the lung. With different ratio depending on the host species, a part of them migrate to the alimentary tract through trachea and pharynx but do not grow to mature adults in the small intestine in most cases. The rest migrate to other organs via blood stream and stay there with keeping infectivity. Humans get severe health problem by migration of the larvae to various organs. This type of infection showing a number of clinical symptoms such as eosinophilia, fever, cough, liver enlargement and pneumonia is known as visceral larva migrans. Other type of infection resulting encephalopathy in the brain is known as neuro larval migrans.

Humans get infection either by accidental ingestion of embryonated eggs contaminated in soil or on raw vegetables, or by ingesting raw or undercooked meat and organs that are parasitized by the parasite larvae. In East Asia, the latter route is considered as main in human infection due to the eating habit.

Although it is important to detect infected animals for the control of this infection, there is no established detection method of infection in animals or contamination in raw meat products. In humans, diagnosis of *A. suum* infection relies mainly on immunological assays for specific antibodies such as ELISA (enzyme-linked immunosorbent assay). However, it is an indirect method to detect *A. suum* infection, and cross-reaction with other parasitic infections is often problematic. Although ELISA may be useful for the diagnosis of *A. suum* infection in animals, it cannot accurately evaluate the existence of larvae in meat products. From the public health point of view,

it is requisite to develop a fast and reliable method for demonstrating *A. suum* larvae contamination in raw meat products. The aim of this study is to develop a sensitive, specific and time-saving real-time PCR assay in order to detect *A. suum* larval contamination in raw meat products.

Materials and Methods

Adult worms of *A. suum*, *Toxocara canis,* and *T. cati* were collected from infected pigs, dogs and cats, respectively. The eggs were removed from the uterus and cultured for maturation. Larvae were then hatched mechanically and isolated. For *A. suum*, larvae migrating to the lung were also collected from experimentally infected rabbits. Besides, adult *A. lumbricoides* were collected from a human patient. All samples were fixed in 70% ethanol and then DNA was extracted.

A TaqMan probe for *A. suum* was designed and a real-time PCR assay was developed using a reported primer set (Pecson *et al.*, 2006) so that a fragment (82 bp) of the ITS1 region was amplified. Standard curve was obtained using the conventional PCR product of *A. suum* ITS1 region.

At first, sensitivity and specificity of the assay was evaluated using the samples obtained. Then the assay was applied on DNA extracted from 1, 2, 5, 10 or 20 isolated *A. suum* larvae and from 500 mg of mouse livers spiked with the same number of the larvae. Finally, mice were experimentally infected with doses of 100, 300 and 900 *A. suum* matured eggs. DNA was extracted from 500 mg of mouse livers which was minced and mixed well at 2 days post-infection. In order to know the number of larvae existed in the livers, the remaining minced livers were digested in pepsin-HCl and the number of larvae collected was counted with being adjusted into 500 mg.

Results

The developed real-time PCR assay could constantly detect *A. suum* DNA up to 10 fg, equaling to 26 copies of ITS1 region. Amplification was not observed with *T. canis* and *T. cati* DNA. However, the assay amplified *A. lumbricoides* DNA. The amplification was observed with *A. suum* DNA not only from isolated single larva but also from mouse liver spiked with single larva. However, the efficiency of amplification was reduced to approximately 2.6 times lower copy number of ITS 1 gene in the samples spiked with liver tissue.

In the experimental infection study, the assay could detect *A. suum* DNA in all livers of experimentally infected mice, except for one infected with 100 eggs. In digestion method, 13.6 and 5.3 larvae were recovered from the liver of mice inoculated with 900 and 300 eggs, respectively. However, no larva was recovered from that of mice inoculated with 100 eggs.

Discussion

The developed assay could amplify *A. suum* DNA from single larva either of isolated or spiked to mouse liver. Although the efficiency for detecting larvae was reduced when it is applied on liver tissue, the sensitivity of the assay was enough for detecting one larva. The reduction of PCR efficiency could be due to the interference by PCR inhibitors, such as heme, and by the excess amount of host DNA originated from liver tissue.

In experimental infection study, no larva was recovered from the livers of 5 mice inoculated with 100 eggs by digestion method. However, the real-time PCR assay could detect *A. suum* DNA in 4 of the 5 mice. This indicates that the real-time PCR assay has higher sensitivity than a conventional digestion method.

Conclusion

The real-time PCR assay with high sensitivity and specificity was developed in this study to detect *A. suum* larval contamination in the liver that has a risk of transmission of *A. suum* to humans. This assay can be applied in a meat inspection procedure, especially for inspecting chicken or cattle liver having white spot lesions – a potential pathological evidence of *A. suum* infection. Therefore, the assay enables us to find infected chickens as well as infected farms, leading to understanding the transmission route of the parasite to animals in the farms and to establishing a preventive method.

備考　論文要旨は、和文にあっては 2，000 字程度、英文にあっては 1，200 語程度とする。