RESEARCH REPORT - No. 1202 FY 2012–2013

Title of research project	Survey of contamination by parasites in livestock and game meat and development of risk assessment method for meat-borne parasitic diseases based on the epizootic and epidemiological characteristics
Research project no.	(1202)
Research period	FY 2012–2013
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【Abstract】

In this study, to evaluate the threats to human health from parasites in livestock meat and game meat in Japan, a risk assessment method (hereinafter, risk map method) was developed based on enzootic and epidemiological data of 14 meat-borne parasites. The data consisted of 1) meat inspection statistics of slaughtered animals (2001-2012), 2) number of human cases reported in the literatures and confirmed by National Institute of Infectious Diseases, 3) receipt data, and 4) prevalence of emerging parasites (*Taenia asiatica*, *Sarcocystis fayeri*, *Kudoa septempunctata*), *Trichinella* nematodes, *Toxoplasma gondii* and lung flukes, which are becoming serious public health issues in Japan.

1) *Taenia asiatica* and *Trichinella* nematodes: A total of 470 pig livers from abattoirs in 3 prefectures in Kanto District were inspected for larval *T. asiatica* (cysticerci). Suspected 83 white spots detected in 89 livers were all negative for *T. asiatica* infection. Experiments using miniature pigs experimentally infected with *T. asiatica* revealed that the parasite developed into small larval cysticerci in the liver at 9 days after infection, but the cysticerci were then died by 20 days post-infection. In *Trichinella*, a total of 24 bear meat from Hokkaido and Honshu were examined by artificial digestion method and real-time PCR assay, but all of the samples tested were negative for *Trichinella* infection.

2) Toxoplasma (*Toxoplasma gondii*): Molecular phylogenetic analysis using 3 genes of *UPRT*, *HP1* and *SAG1* revealed that 18 *T. gondii* clones isolated in Okinawa could be classified as G1 and G2, which were unique for Okinawa, and cosmopolitan Type II and III. We also attempted to examine anti-*T. gondii* antibodies in livestock and poultry in Honshu Island and found that 7.3% and 5.2% of the cattle and pigs were positive, respectively. Our findings therefore suggest that toxoplasmosis in Japan can be attributed to the consumption of infected raw beef.

3) Lung flukes (*Paragonimus* spp.): To evaluate the contamination levels of human-infecting *Paragonimus* larvae in wild boar and deer meat, 22 and 4 samples of wild boar and wild deer meat, respectively, from Kyushu were examined. *Paragonimus westermani* larvae were detected in 7 wild boar meat from Oita and Kagoshima prefectures, but all the deer meat samples were negative for *Paragonimus* larvae. We also found that *P. westermani* larvae stored at −18ºC for 24 hours had completely lost the ability to infect recipient mice. We should therefore provide health education, particularly to wild-meat suppliers, to emphasize the risk of paragonimiasis due to the consumption of meat without prior deep freezing.

4) *Sarcocystis fayeri*: Food poisoning associated with consumption of raw horse meat, "*basashi*" is caused by

Sarcocystis fayeri, but little information on the prevalence of *S. fayeri* infection in domestically produced horses has been reported to date. We quantitatively estimated *S. fayeri* DNA in the diaphragm tissue (5 samples/horse) of 300 heavy draft horses that were born in Japan and slaughtered in Kumamoto. Real-time PCR assay showed that only 3 (0.2%) of the 1,500 diaphragm samples contained the same amount of *S. fayeri* DNA that was present in the *basashi*-associated food poisoning case. The findings revealed that sarcocyst tissue concentrations were relatively low in the meat of domestic draft horses.

5) *Kudoa septempunctata*: We quantitatively estimated the incidence of *K. septempunctata* in domestically farmed "*hirame* (Olive flounder)". A total 115 "*hirame*" samples from Oita (70), Ehime (20) and Mie (25) prefectures were examined by real-time PCR, all of the samples tested were negative for *K. septempunctata.* The prevalence of *K. septempunctata* in farmed *hirame* in Japan was considered to be relatively low. The incidence of food poisoning cases caused by *K. septempunctata* decreased in 2013.

Based on the epizootic and epidemiological characteristics of the 14 meat-borne parasites including parasites described above, a semi-quantitative risk map method was developed and relative risks affected by these parasites were visualized. *Toxoplasma gondii* was placed in the unacceptable risk zone due to high risk for newborn babies. *Taenia asiatica*, *S. fayeri* and *K. septempuctata*, which are all emerging parasites, were mapped in the risk-reduced zones. The lung fluke was mapped in the socially acceptable risk zone due to a small number of patients affected. The risk map methodology developed in this study is intended to evaluate the threats to human health associated with parasite contamination in livestock meat and game meat, but it is applicable to compare changes in risk associated with previous and current food poisoning incidents caused by the parasites and also could be considered useful for assessing the effectiveness of mitigation measures implemented by public health authorities against food-borne parasitic diseases.