Outbreak of *Trichinella* T9 Infections Associated with Consumption of Bear Meat, Japan

Technical Appendix

**Method for Performing ELISA Using Excretory–Secretory Antigens from *Trichinella spiralis***

ES products using as antigen for an ELISA were prepared from *T. spiralis* (ISS413) muscle-stage larvae according to the method of Gómez-Morales et al. (1). In brief, muscle-stage larvae were isolated by pepsin-HCl digestion from mice at 30 days post-infection. The larvae were washed 3 times in phosphate-buffered saline (PBS) and then incubated in Dulbecco’s modified Eagle’s medium at 37°C for 18 h. The medium was filtered through a 0.2-µm YM-5 filter and concentrated 100 times in an Amicon pressure concentrating chamber (Amicon, Inc., Billerica, MA, USA).

The ELISA was performed as reported in our previous study (2,3) with slight modifications. In brief, 96-well microtiter plates (MaxiSorp, Nalge Nunc International, Tokyo, Japan) were sensitized with ES antigen at 5 µg/ml in 0.05 M bicarbonate buffer, pH 9.6 (100 µl/well) for 3 h at 37°C and overnight at 4°C. After the microplates were washed 3 times with PBS, they were blocked with 150 µl of PBS containing 2% bovine serum albumin (BSA) for 2 h at 37°C. After washing with PBS-0.05% Tween 20, the microplates were probed with a diluted human serum sample (1:200–1:25,600, 100 µl/well) in PBS containing 1% BSA for 1 h at 37°C. After washing, 100 µl of 1:10,000-diluted goat anti-mouse IgG (Fab-specific) peroxidase-conjugate (Sigma Chemical Co., St. Louis, MO, USA) was incubated for 1 h at 37°C. For color development, 2–2’-azono-bis (3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co.) was added to each well as a substrate (0.3 mg/ml, 100 µl/well), and the reaction was terminated after 60 min by adding 50 µl of 1.25% sodium fluoride per well. The absorbance at 414 nm was monitored with a Multiskan JX plate reader (Labsystems, Helsinki, Finland). Since “3 × the A414 means of 1:200-diluted negative sera from 100 healthy persons” was larger than “the means plus 3 standard deviations,” “3 × the A414 mean of the negative sera” was determined as the cutoff.
value in further experiments, resulting in a value of 0.148 for our ELISA test. Each plate contained four positive and four negative reference serum samples. Three-well repeats were measured for each diluted serum sample. The ELISA titers were shown as the highest serum dilution that yielded an \( A_{414} \) greater than the cutoff point.

References


Technical Appendix Figure. The relationship between the date on which the infected bear meat was consumed and the occurrence of trichinellosis. A confirmed case was defined as patients with 1) a history of consuming raw bear meat; 2) clinical symptoms compatible with trichinellosis; and 3) serologic evidence of trichinellosis. A probable case was defined as patients with 1) a history of consuming raw bear meat; 2) clinical symptoms compatible with trichinellosis; and 3) negative serologic results. Asterisk indicates that the patient consumed bear meat from a different block of meat from the other case-patients.