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First two confirmed cases of *Dibothriocephalus nihonkaiensis* in Southeast Asia (Singapore)

Sir,

Recent reports suggest that there has been an increase of diphyllobothriosis globally, particularly in developed countries with high standards of sanitation.[1–5] Diphyllobothriosis is an intestinal parasitosis acquired by consumption of raw or undercooked fish harbouring the infective plerocercoid larvae of *Dibothriocephalus* and *Diphyllobothrium* species. More than 50 species of broad fish tapeworm have been described, of which *Dibothriocephalus latus* (previously known as *Diphyllobothrium latum*) and *Dibothriocephalus nihonkaiensis* (previously known as *Diphyllobothrium nihonkaiense*[6] have been considered the principal human pathogens in Europe and Northeast Asia, respectively. *Dibothriocephalus latus* uses freshwater fish such as pike, perch, burbot and char as the second intermediate hosts, while *D. nihonkaiensis* exploits anadromous Pacific salmons.[3] The consumption of raw Pacific salmon may be a risk factor for *D. nihonkaiensis* infection.[2]

Dibothriocephalus nihonkaiensis is almost exclusively found in Japan; however, clinical cases have been subsequently reported in South Korea, China, European countries and the North Pacific coast of North America in the past decade, possibly due to the globalisation of the fresh or frozen Pacific salmon trade and also due to awareness and better diagnostic techniques. Singapore imports over 90% of the fish consumed in the country.[7] All salmon products consumed in Singapore are imported. Atlantic (Norwegian) salmon is widely available in local supermarkets. Pacific salmon sourced from various countries is also available in specialty stores and food outlets locally.

We present the first two cases of *D. nihonkaiensis* diagnosed and reported from Singapore. To our knowledge, these are the first cases of *D. nihonkaiensis* confirmed in Southeast Asia based on the DNA sequences of mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*).

The first case was a 46-year-old asymptomatic woman, who sought medical attention after she had passed a worm per rectum. In the laboratory it was found to be a tapeworm strobili, which measured approximately 2.85 m long. The scolex was not found. There were 835 segments and the proglottids measured 2.0–2.5 mm long × 5.0–7.5 mm wide after fixation in 10% neutral buffered formalin.

Two weeks later, a 29-year-old woman similarly sought medical attention after passing a worm. Specimen submitted (Fig. 1A) was again identified to be a section of strobila

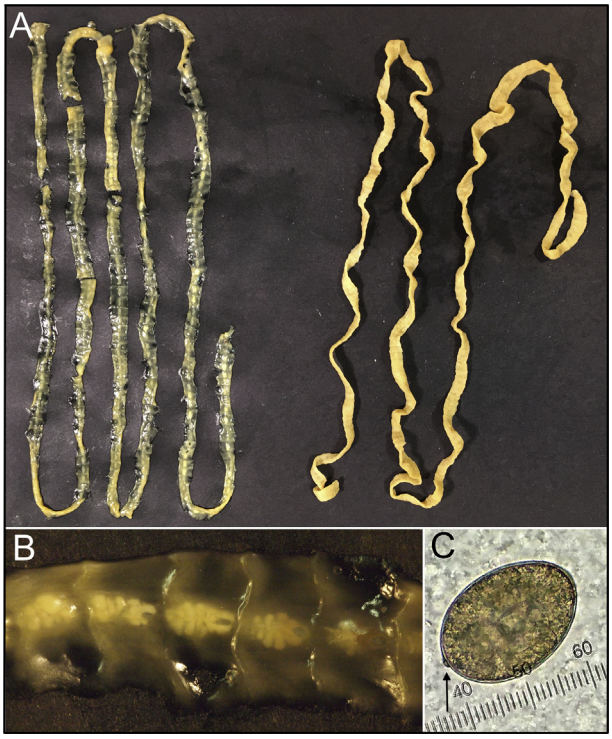

Fig. 1 (A) The specimen was a section of strobila without scolex. The strobila was approximately 1.5 m long. There were 748 segments and the proglottids measured 2.0–2.5 mm long × 5.0–7.5 mm wide after fixation. The segment on the right was soaked in formalin. (B) Close up view of proglottids showing rosette-shaped uterus in the centre of each. (C) *Dibothriocephalus nihonkaiensis* egg in an unstained wet mount. The egg measured approximately 55 µm × 90 µm. Note the apical knob at the abopercular end (arrow).

without scolex. The strobila was approximately 1.5 m long. There were 748 segments and the proglottids measured 2.0–2.5 mm long × 5.0–7.5 mm wide after fixation. Close up view of the proglottids showed rosette-shaped uterus in the centre of each proglottid (Fig. 1B). Eggs measured approximately 55 µm × 90 µm and had an apical knob at the abopercular end (Fig. 1C).

Both patients regularly consumed *sashimi* and *sushi*, including raw salmon. The patients were treated with the anthelminthic praziquantel. Both patients did not report further passing of identifiable worm segments per rectum after praziquantel administration.

While broad fish tapeworms are generally identified to a genus level based on morphological characteristics, accurate determination of the species is only possible by molecular analysis. It is probable that most diphyllobothriosis cases originally attributed to *D. latum* may have been caused by *D. nihonkaiensis* tapeworms when morphology alone was used. DNA was extracted from the proglottids using DNeasy Blood and Tissue kit (Qiagen, Germany). Polymerase chain reaction (PCR) was performed using Qiagen multiplex PCR kit with forward primer 5′-ACAGTGGGTTTA-GATGTAAAGACGGC-3′ and reverse primer 5′-AGCTA-CAACAAACCAAGTATCATG-3′ for the amplification of a 249 bp fragment of the *cox1* gene as previously published.[8] As the tapeworm samples were stored in 10% neutral buffered formalin, a protocol yielding a short amplicon was chosen to minimise the effect of DNA fragmentation due to formalin. DNA sequences from both samples most closely matched that of *D. nihonkaiensis* (GenBank accession number LC070678).

Diphyllobothriosis is a rare infection in Singapore. These cases demonstrate the global spread of a food-borne pathogen as a result of global food trade and the increasing popularity of *sashimi* and *sushi*. Although species identification is not essential for the effective treatment of diphyllobothriosis, it is important for the purpose of epidemiology and food safety. Due to the worldwide transport of raw fish including salmon, various unreported broad fish tapeworm species can be introduced into Singapore. Species level identification will aid in the determination of the possible sources of plerocercoids. This in turn will help local food safety surveillance and practices.

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Yersinia pseudotuberculosis bacteraemia: a diagnostic dilemma in the era of MALDI-TOF mass spectrometry

Sir,

A 73-year-old woman presented febrile, hypotensive and tachycardic with profuse diarrhoea whilst receiving oxaliplatin chemotherapy for metastatic colorectal carcinoma and letrozole therapy for synchronous breast and vulval carcinoma. Additional history included type 2 diabetes mellitus. She had not recently travelled, had no regular animal contact and had an absence of flea bites, although a mouse plague was contemporaneously afflicting the local region.

After 28 hours incubation, peripheral and PICC anaerobic blood cultures (BACTEC; BD, USA) flagged with Gram-negative bacilli with bipolar staining. Identification by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Australia) on early growth from the chocolate-agar (bioMérieux, Australia) incubated in 5% carbon dioxide at 37°C was performed using the Bruker Standard Taxonomy (version 7.0) and Security Relevant databases. A reliable identification was not obtained with acceptable scores for both *Yersinia pestis* (2.24) and *Yersinia pseudotuberculosis* (2.23), with scores greater than 2.0 considered consistent with species-level identification.

Given the public health and biosecurity significance of *Y. pestis*, a definitive identification was urgently sought. A positive urease test was suggestive for *Y. pseudotuberculosis*; however, the 2 hour peptone broth motility at 25°C was negative. The VITEK 2 (bioMérieux) supported an identification of *Y. pseudotuberculosis* using phenotypic characteristics with a 99% probability (Bionumber 4005711300100210). 16S ribosomal RNA gene sequencing obtained a 1, 147 base pair sequence; however, it was unable to distinguish between the two species as the obtained sequence was almost identical to both the *Y. pestis* (GenBank CP019708.1) and *Y. pseudotuberculosis* (Genbank HG326181.1) reference sequences.