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Human case of *Onchocerca dewittei japonica* infection in Fukushima, Northeastern Honshu, Japan

Masako Fukuda^{a,*}, Shigehiko Uni^{b,c}, Takahiro Igari^{d,e}, Yasufumi Utsumi^f, Yasushi Otsuka^g, Jun Nakatani^h, Shoji Uga^c, Tatsuru Haraⁱ, Hideo Hasegawa^j, Hiroyuki Takaoka^k

^a Institute for Research Promotion, Oita University, Oita 879-5593, Japan

^b Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

^c Department of Public Health, Faculty of Nursing, Kobe Women's University, Kobe 650-0046, Japan

^d Department of Orthopaedic Surgery, Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan

^e Department of Orthopaedic Surgery, Ohara General Hospital, Fukushima 960-8611, Japan

^f Department of Diagnostic Pathology, Ohara General Hospital, Fukushima 960-8611, Japan

^g Research Center for the Pacific Islands, Kagoshima University, Kagoshima 890-8580, Japan

^h Agriculture Research Center, National Agriculture and Food Research Organization, Tsukuba, Ibaraki 305-8666, Japan

ⁱ Department of Parasitology, Kurume University School of Medicine, Kurume 830-0011, Japan

^j Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita 879-5593, Japan

^k Tropical Infectious Diseases Research and Education Centre (TIDREC), University of Malaya, 50603 Kuala Lumpur, Malaysia



ARTICLE INFO

Keywords:

Black fly

Filarial parasite

Japanese wild boar

Onchocercosis

Vector-borne disease

Zoonosis

ABSTRACT

A 73-year-old man living in Kawamata-machi, Fukushima Prefecture, Northeastern Honshu, Japan, visited a hospital with complaints of a subcutaneous swelling that had developed on the back of his left hand. The nodule was surgically removed from the vagina fibrosa tendinis of his left forefinger. Based on the histopathological characteristics, the causative agent of this nodule was identified as a female *Onchocerca dewittei japonica* (Spirurida: Onchocercidae). The species identification was confirmed by *cox1* gene sequencing of the worm tissues from paraffin-embedded sections of the nodule. Although 11 cases of zoonotic onchocercosis have previously been recorded in Kyushu and Western Honshu, Japan, the present findings represent the first human case of infection with *O. dewittei japonica* in Northeastern Honshu, Japan.

Zoonotic onchocercosis is an emerging human infection caused by animal parasitic *Onchocerca* species, which are transmitted by black flies or biting midges. Recently, the numbers of infected individuals have increased worldwide. A total of 36 cases in humans have now been reported, with 13 in North America (11 in the USA and two in Canada), 11 in Japan, six in Europe, three in Turkey, and one each in Kuwait, Tunisia, and Iran. Five *Onchocerca* species (*O. gutturosa*, *O. cervicalis*, *O. dewittei japonica*, *O. jakutensis*, and *O. lupi*) have been identified as the causative agents [1–11]. In all the Japanese cases, the causative agent was *O. dewittei japonica* [1,8–12]. This filarial worm is naturally parasitic in the Japanese wild boar (*Sus scrofa leucomystax*). In Oita, Kyushu, its transmission vector was found to be the anthropophilic, zoophilic black fly, *Simulium bidentatum* [13].

To effectively plan public health strategies to prevent the transmission of zoonotic filariae to humans, it is essential to record the geographical distribution of the disease, along with the causative agents and vectors, systematically. Here, we report on a new clinical case of

zoonotic onchocercosis caused by *O. dewittei japonica* in Northeastern Honshu, Japan, where human cases have not been previously detected.

A 73-year-old man living in Kawamata-machi, Fukushima Prefecture, Tohoku Region, Japan (Fig. 1), visited Ohara General Hospital, Fukushima City, complaining of a painless subcutaneous nodular swelling on the back of his left hand in January 2017. The nodule adjacent to the vagina fibrosa tendinis of the left forefinger was surgically removed in March 2017, and his symptoms subsided soon after the surgery.

The excised mass (2 × 2 cm) was fixed in 10% buffered formalin for several hours and then embedded in paraffin according to routine histopathological procedures. Sections were stained with hematoxylin and eosin. By microscopy, the mass was found to be a granuloma containing nematode parts. The nematode was 169–223 μm wide with a four-layer thick cuticle (11–27 μm in thickness). The cuticle had external transverse ridges that were noticeably triangular (height, 9–16 μm; width, 20–28 μm) in the longitudinal sections (Fig. 2a). The distance between

* Corresponding author.

E-mail address: mfukuda@oita-u.ac.jp (M. Fukuda).

<https://doi.org/10.1016/j.parint.2019.101943>

Received 17 October 2018; Received in revised form 7 June 2019; Accepted 16 June 2019

Available online 17 June 2019

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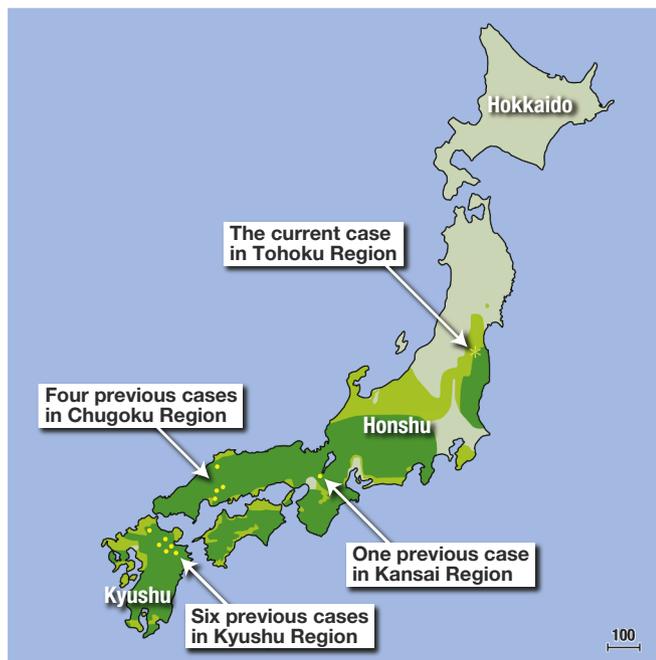


Fig. 1. Geographical distribution of human cases of zoonotic onchocercosis in Japan. The asterisk denotes the current case in Tohoku Region. Yellow dots denote previous cases. Regions where case(s) have been reported are indicated by the rectangular captions. Dark green indicates the habitat of the Japanese wild boar (*Sus scrofa leucomystax*) in 1978, light green indicates their newly confirmed habitats up until 2014, and pale green indicates areas with no wild boars [17]. Scale bar, km. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adjacent ridges was 112–251 μm . There were no inner striae in the cuticle's middle layer. The intestine (diameter, 9–13 μm) and four uterus sections, in which microfilariae were not visible, were observed in the slightly oblique transverse section of the worm (Fig. 2b).

The patient lived in a mountainous area inhabited by wild boars. As a farmer he used to hunt wild boars with his dogs before the nuclear power plant accident in Fukushima in 2011. His dogs had no history of filariasis. He had never travelled outside of Fukushima Prefecture.

In Japan, eight named *Onchocerca* species (*O. cervicalis*, *O. dewittei japonica*, *O. eberhardi*, *O. gutturosa*, *O. lienalis*, *O. skrjabini*, *O. suzukii*, and *O. takaokai*) and one unnamed species (*Onchocerca* sp. type A) have been recorded in non-human animals [14]. Based on the morphological characteristics of the filarial sections (i.e., thin body, thick cuticle, prominent outer transverse ridges, relatively long adjacent ridge distances, and the lack of inner striae in the cuticle's middle layer), the causative agent of the patient's infection was diagnosed as a female *O. dewittei japonica*.

For confirmation, we conducted molecular typing based on 125 parasite tissue samples scraped from four 10 μm -thick unstained histological sections. DNA extraction, PCR amplification with CO1f-CO1fR primers, and nucleotide sequence analysis were performed as described previously for the case from Shimane Prefecture [9], except for the number of PCR cycles, which was 50 in this study. The primers were used to amplify short sequences (expected size: 155 bp) because of the small amount of extracted DNA and the possibility of DNA degradation in the formalin-fixed, paraffin-embedded tissues. The genetic

analysis was based on a 110 bp (excluding primers) fragment of the mitochondrial *cox1* gene using MEGA6.06 [15]. The phylogenetic tree constructed using the neighbor-joining method was based on the *cox1* sequences from the worm from this case and eight *Onchocerca* taxa in GenBank, including *Onchocerca* sp. type A, *Cercopithifilaria longa* and *Loxodontofilaria caprini* were selected as the outgroups. The accession numbers of the *cox1* sequences are shown in Fig. 2c.

The nucleotide sequences from the causative worm in the current case were identical to those of *O. dewittei japonica* from a wild boar [16] and five previous human cases in Hiroshima, Fukuoka, and Shiga [8–11]. The sequences from the six human cases differed by one of the 110 nucleotide pairs from those of *O. dewittei japonica* from the human case in Shimane [9] (Fig. 2c). The phylogenetic analysis indicates that *O. dewittei japonica*, the causative species of zoonotic onchocercosis in Japan, is distinguishable from the other species by the high bootstrap values (Fig. 2c).

With the *O. dewittei japonica* infections, a single subcutaneous nodule was the only major recognizable clinical sign in each of the 12 human cases reported in Japan. The signs manifested themselves in a hand, an arm, or a leg in nine cases, and in the retroauricular, infraclavicular or temporal region in the other three [9]. The ocular manifestations reported for patients with *O. lupi* were not found in the patients with *O. dewittei japonica* [4,6].

Wild boars are common in Kyushu and Western Honshu, and their distribution is expanding annually towards Northeastern Honshu (Fig. 1). Decreased annual snowfall owing to global warming, increased unused rice fields owing to a decrease in the young workforce in rural areas, and a decline in the number of hunters in a more aged population have led to wild boars migrating for food [9,17]. Notably, wild boars have propagated in the rural areas of Fukushima, because many residents have evacuated their home villages since the nuclear power plant accident caused by the Great East Japan Earthquake in 2011. High infection rates of *O. dewittei japonica* in wild boars were reported in Oita, Kyushu and Shimane in Western Honshu, where human infections have previously been detected [1,9]. A high prevalence of *Dirofilaria immitis* in dogs increases the risk for filarial infection in the human population [18]. Similarly, the current study suggests that *O. dewittei japonica* infections are prevalent in wild boars in Fukushima.

Further studies are needed to determine the vector of zoonotic onchocercosis in Fukushima, although *S. bidentatum* is likely to be involved in the transmission because it is one of the most abundant and widespread black fly species in Kyushu and Honshu [14,19]. Overall, our study presents explicit evidence that human infections with *O. dewittei japonica* can occur even in the northeastern region of Honshu, although its transmission may be limited to a few summer months when air temperatures exceed 17 $^{\circ}\text{C}$, the critical air temperature needed for *O. volvulus* larvae to develop in the black fly vector [20].

Declarations of interest

None.

Acknowledgements

We are grateful to Mr. Shuji Imi, Oita University, for his assistance. This work was partly supported by JSPS KAKENHI (Grant Numbers 15K07781 and 19K06421).

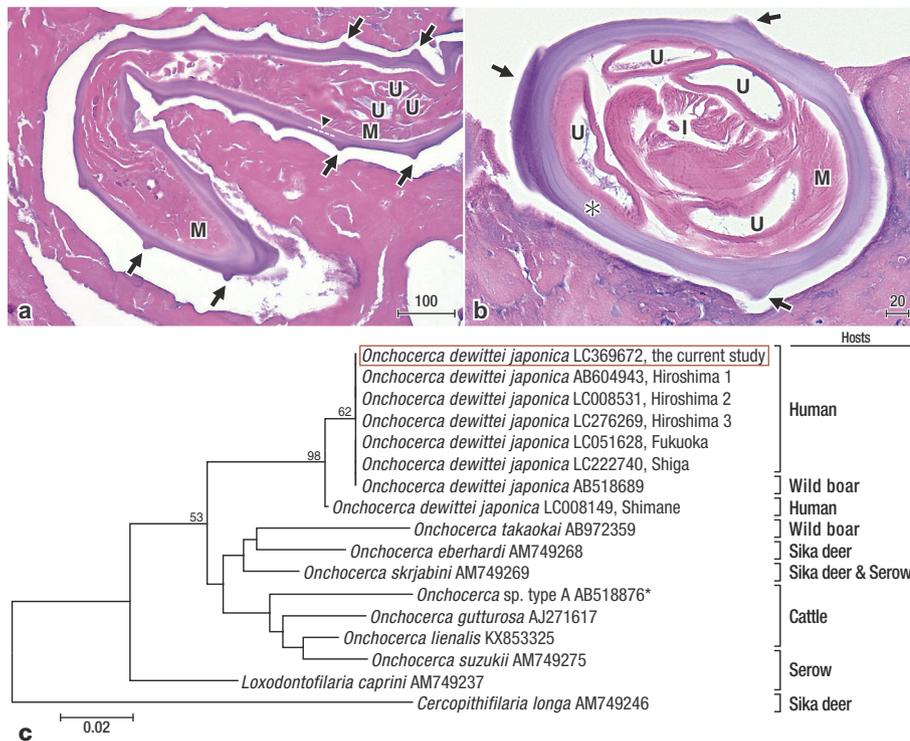


Fig. 2. Histopathological characteristics of the female *Onchocerca dewittei japonica* from the nodule excised from the patient (a, b) and molecular identification of the parasitic worm (c). a) Longitudinal section: arrows, prominent triangular ridges; white line with arrowhead, middle layer of the cuticle without inner striae; M, muscle layer; and U, uterus. Hematoxylin and eosin (HE) staining. Scale in μm . b) Slightly oblique transverse section: arrows, elevation of the cuticle, indicating transverse ridges; *four-layered thick cuticle; I, intestine; M, muscle layer; and U, uterus. HE staining. Scale in μm . c) Phylogenetic tree based on the partial mitochondrial *cox1* gene sequences from *Onchocerca* spp. using the neighbor-joining method. Hiroshima, Fukuoka, Shiga, and Shimane are the Japanese localities where human infections have previously occurred. The vertebrate host for each filarial species is indicated. *This sequence was obtained from a third-stage larva of an unnamed bovine filaria (*Onchocerca* sp. type A) in *Simulium bidentatum* collected in Oita [13]. Numbers at the nodes are the bootstrap confidence values after 500 replications. Values > 50% are shown. The scale-bar indicates the distance in substitutions per nucleotide.

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