ZOONOTIC ONCHOCERCIASIS CAUSED BY A PARASITE FROM WILD BOAR IN OITA, JAPAN

A COMPREHENSIVE ANALYSIS OF MORPHOLOGICAL CHARACTERISTICS OF THE WORMS FOR ITS DIAGNOSIS

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Summary:

Histological examination of a nodule removed from the back of the hand of a 58-year-old woman from Oita, Kyushu, Japan showed an *Onchocerca* female sectioned through the posterior region of the worm (ovaries identifiable) and young (thin cuticle). Six *Onchocerca* species are enzootic in that area: *O. gutturosa* and *O. lienalis* in cattle, *O. suzukii* in serows (*Capricornis crispus*), *O. skrjabini* and an *Onchocerca* sp. in *Cervus nippon nippon*, and *O. dewittei japonica* in wild boar (*Sus scrofa leucomystax*). Diagnostic characters of female *Onchocerca* species, such as the cuticle and its ridges, change along the body length. Tables of the histologic morphology of the mid- and posterior body-regions of the local species are presented. In addition, it was observed that transverse ridges arose and thickened during the adult stage (examination of fourth stage and juvenile females of *O. volvulus*). The specimen described in this report, with its prominent and widely spaced ridges, was identified as *O. d. japonica*. Four of the 10 zoonotic cases of onchocerciasis reported worldwide were from Oita, three of them being caused by *O. d. japonica*, the prevalence of which in local wild boar was 22 of 24 (92 %).

KEY WORDS : zoonosis, Japan, *Onchocerca dewittei japonica*, wild boar.

Résumé : ONCHOCERCOSE ZOONOTIQUE À OITA, JAPON AVEC LA DESCRIPTION D'UN QUATRIÈME CAS

Une femme de 58 ans vivant à Oita, Kyushu, Japon, a un nodule sur le dos de la main; l'histologie révèle la présence d'une onchocerce femelle, jeune (cuticule mince), sectionnée dans la région postérieure (ovaires identifiés). Six espèces sont présentes dans la région: *O. gutturosa* et *O. lienalis* chez le bétail, *O. suzukii* chez le serow *Capricornis crispus*, *O. skrjabini* et *Onchocerca* sp. chez *Cervus n. nippon* et *O. dewittei japonica* chez le sanglier, *Sus scrofa leucomystax*. Un tableau de leurs caractères discriminatifs histologiques est effectué, en distinguant les régions moyenne et postérieure du corps. Des stades 4 et des femelles juvéniles d'onchocerce sont aussi observés (*O. volvulus*); elles montrent que les côtes transversales se forment et s'épaississent durant le stade adulte. Le spécimen étudié, avec ses côtes transversales remarquablement saillantes et espacées, peut être identifié à *O. d. japonica*. Quatre des 10 cas d'onchocercose zoonotique connus dans le monde proviennent d'Oita et trois d'entre eux sont dus à *O. d. japonica*; 92 % des 24 sangliers examinés dans la région sont parasités par cette onchocerce.

MOTS CLÉS : zoonose, Japon, *Onchocerca dewittei japonica*, sanglier.

Zoonotic onchocerciasis is a rare infection in humans: a total of nine cases has been previously described worldwide in the last 40 years (Burr *et al.*, 1998; Pampiglione *et al.*, 2001; Takaoka *et al.*, 2001); interestingly, three of them were reported from the same locality, Oita, Kyushu, Japan; one case was described by both Beaver *et al.* (1989) and Hashimoto *et al.* (1990), and the others by Takaoka *et al.* (1996, 2001). The new case reported here also occurred

in this region, in a patient who has not travelled outside Kyushu. These four cases suggest the existence of local conditions favorable for the transmission of animal *Onchocerca* species to humans.

Oita is a region with cultivated areas, cattle ranches, and wild-life sanctuaries (Mt. Sobo, 1,756 m high) that shelter the wild ungulates: serows (*Capricornis crispus* Temminck), sika deer (*Cervus nippon nippon* (Temminck)) and wild boar (*Sus scrofa leucomystax* Temminck). Studies done during the past fifteen years (Takaoka *et al.*, 1989; Takaoka & Bain, 1990; Yagi *et al.*, 1994; Takaoka, 1994; Uni *et al.*, 1997; Takaoka, 1999; Uni *et al.*, 2001) have shown the diversity of *Onchocerca* species enzootic in Oita. In addition to two cosmopolitan parasites from cattle, *O. gutturosa* Neumann, 1910, and *O. lienalis* Stiles, 1892, four other species have been identified from wild ungulates in Oita, of which one has not yet been described (Uni *et al.*, in preparation): *O. suzukii* Yagi, Bain & Shoho, 1994, from serow, which may also be transfused in cattle (Uni *et al.*, 1997), *O. skrjabini* Rukhlyadev, 1964, a palearctic species from cervids (Bain & Schulz-Key, 1974; Barùs

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Fig. 1. – *Onchocerca* species in Oita, Japan, histologic sections. A to C, from the patient. A: Tumor showing the two worm sections and the surrounding tissue reaction. B: Transverse section of female worm showing the two-layered cuticle, tall somatic cells, marked lateral chords, four sections of ovaries (one or two reproductive ducts), and the small intestine. C: Longitudinal section of female worm showing marked transverse ridges on the external surface of the cuticle. D to F, Female body cuticle of three *Onchocerca* species from wild ungulates, longitudinal sections. D: *O. dewittei japonica* from wild boar. E: *O. skrjabini* from *Cervus n. nippon*. F: *Onchocerca* sp. from *Cervus n. nippon*.

Scales bars in μm : A, 200; B, 30; C-F, 50.

& Koubek, 1993), and the closely related species, *Onchocerca* sp. Uni, Bain & Takaoka, both from sika in Oita, and *O. dewittei japonica* Uni, Bain & Takaoka, 2001, from wild boar.

Definitive identification was possible of the causative agent of one of the human cases as *O. d. japonica* (Takaoka *et al.*, 2001) because the male filaria had the cuticular longitudinal crests characteristic of the male of this species (Uni *et al.*, 2001). One specimen from the second human case (Takaoka *et al.*, 1996), reported before the discovery of *O. d. japonica*, was later shown to correspond to *O. d. japonica* (Takaoka *et al.*, 2001). The filaria of the first case (Beaver *et al.*, 1989; Hashimoto *et al.*, 1990) had not been identified because only transverse sections were available for examination; however, the possibility was ruled out that it was the *Onchocerca* species from a serow discovered earlier (Suzuki *et al.*, 1982), but not described at that time.

As part of the identification of the fourth case of zoonotic onchocerciasis in Oita reported here, we have summarized the characteristics of local *Onchocerca* species. These characteristics are visible in histologic sections and are based on descriptions of mature worms. We took into consideration that cuticular and hypodermal characteristics change along the length of the female. Moreover, the worm identified in this case report was apparently young, so we set out to collect data on the development of the cuticle and its ornamentation from the specimens of *O. volvulus* (Leuckart, 1893) material deposited in museum collections, for use for this diagnosis and identification of *Onchocerca* spp. in histologic sections.

MATERIALS AND METHODS

A nodule was detected in early April 2003 in a 58-year-old woman living in Notsuharu, Oita Prefecture, in southwestern Japan. The subcutaneous swelling was on the back of the left hand; the lesion was inflamed and tender on April 10, 2003. The nodule, about 2.5 cm in diameter, was biopsied at Notsuharu Hospital on the same day, fixed in 10 % formalin, sectioned, and stained with hematoxylin-eosin. Two histologic sections of the nodule available for study contained a nematode surrounded by granulomatous tissue infiltrated with many lymphocytes, histiocytes (= epithelioid cells), and eosinophils (Fig. 1A-C). The remains of the nodule (collection number MNHN 441 SE) were dissected in lactophenol for recovery of the remaining worm segments, but none was found. Laboratory examination and tests of the patient's blood and serum included a complete blood cell count, including a differential blood count, the activities of aspartate transaminase, alanine aminotransferase, and

lactate dehydrogenase, and the C-reactive protein level. In addition, the patient's serum taken on April 16, 2003 was tested by ELISA (Korenaga *et al.*, 1983) for *O. gutturosa* antigens. The sera of 13 Guatemalan people giving positive results for *O. volvulus* and those of 12 Japanese people giving negative results for the same worm were used as controls.

For comparative studies, we used histologic sections of *O. d. japonica* from wild boar, coll. number MNHN 442 and 443 SE and coll. number Osaka City University Medical School (OCUMS) 00133-1A (Fig. 1D); *O. skrjabini* from sika, coll. number OCUMS 02786-1 (Fig. 1E); *Onchocerca* sp. Uni, Bain & Takaoka, 2003 from sika, coll. number OCUMS 20249-3 (Fig. 1F); whole specimens of *O. volvulus* female fourth-stage larvae (no vulvar opening) recovered by H. J. Winkhardt in 1985 from an experimentally infected Syrian hamster, *Mesocricetus auratus*, coll. number MNHN, 310 ED; and two female young adults (vulva open), isolated from human nodules by H. Schulz-Key in 1982, coll. number MNHN, 444 SE.

The following histologic and morphologic characteristics of adult worms were taken from a key of *Onchocerca* species (Bain, 1981): (i) cuticle: thickness, number of layers, external ridges (spacing, height, and width at base), internal striae, longitudinal grooves, and lateral thickenings; (ii) symmetry of the body; (iii) hypodermal lateral chords: height and width at base; (iv) muscles: thickness, approximate number per quadrant; and (v) diameters of the intestine and the body. The morphologic characteristics of the midbody region were taken from published descriptions; for the posterior region, which has not been described in detail, we measured several published figures (Bain & Schulz-Key, 1974; Bain, 1975; Bain *et al.*, 1978; Uni *et al.*, 2001).

RESULTS

Blood and chemistry tests were within reference limits except for eosinophilia (6.5 %). The patient's serum reacted with the *O. gutturosa* antigen. The optical density by ELISA for *O. gutturosa* antigen was as follows: 1.38 in the patient, 0.0275 (0.010 and 0.126; median with 25th and 75th percentiles) in Japanese subjects (negative controls) and 0.95 (0.51 and 1.90) in Guatemalan subjects (positive controls).

DESCRIPTION OF WORM FROM THE PATIENT

Only two histologic sections of the worm, one transverse (Fig. 1B) and the other oblique (Fig. 1C), were available for study. The description of the worm is as follows (Table I): dorsoventral symmetry of body, dia-

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	Beaver <i>et al.</i> 1989	Takaoka <i>et al.</i> 1996	Present study	Takaoka <i>et al.</i> 2001	Worm sections of <i>O. d. japonica</i> in tissues of natural host*	
Worm sex	Female °	Female	Female	Male	Female, uterine level	Female, vaginal level °
Transverse ridges	Present	Present	Present	–	Present	Absent
Longitudinal grooves or crests	Absent	Absent	Absent	Present	Absent	Absent
Dorsoventral symmetry	Yes	Yes	Yes	Yes	Yes	Yes
Cuticle laterally thickened	No	No	No	–	No	No
Internal transverse striae	–	Absent	Absent	–	Absent	Absent
Cuticle thickness**	14-40 °°	15-30	4-7	12-17	8-32	2.5
Outer <i>vs.</i> inner layer thickness**	Thicker than 9-11	Equal	Equal	–	Equal	–
Ridge section	–	Sharp	Sharp	–	Sharp	–
Ridge height/width**	–	11/22	5-7/14	–	7-13/12-17	–
Inter-ridge space**	–	67-105	40-80	–	110-150	–
Lateral chord height/width**	NR	16-21/26-52	22-24/16-20	15/30	10/45	12/11
Muscle height**	30	30	18-30	15	45	12
Muscle number per quadrant	5	7-12	5-7 ?	NR	7-12	6
Section diameter**	190-238	182-260	80-100	70-110	150	70-80
Intestine diameter**	5-20	15-22	12-22	12	25	15
Worm identification	? <i>O. d. japonica</i>	<i>O. d. japonica</i>	<i>O. d. japonica</i>	<i>O. d. japonica</i>		
Sex & age of patient	F, 2 years old	F, 57 years old	F, 58 years old	F, 52 years old		

*Unpublished data. **Measurements in micrometers. NR, not reported. °Transverse sections only. °°Including ridges and confirmed by Hashimoto *et al.*, 1990. F: woman.

Table I. – Morphologic characteristics of worm sections from the 4 human cases in Oita, compared with histologic sections of *O. d. japonica* from *Sus scrofa leucomystax*.

meter 80-100 μm . Cuticle: 4-7 μm thick, composed of two layers equal in thickness; cuticle thicker sublaterally because the transverse section crossed through part of the ridges; external surface with prominent, sharp transverse ridges spaced 40-80 μm apart, thickness of ridges twice that of cuticle; internal cuticular layer without transverse striae; longitudinal grooves absent. Lateral chords 22-24 μm thick and up to 20 μm wide. Thin hypodermis at base of muscles. Somatic muscles of the polymyarian-coelomyarian type, muscle cells about 18-30 μm thick, apparently five per quadrant. Genital tract present only in the transverse section that contained four small sections identified to ovaries. Intestine, 12-22 μm in diameter.

HISTOLOGIC CHARACTERISTICS OF *ONCHOCERCA* SPECIES FROM OITA

Key characteristics are shown in Fig. 2. Comparisons of the midregion and the posterior region are separate (Table II). The dorsoventral asymmetry of the body calls for comment. Such asymmetry is observed only in *O. gutturosa*; it has been described by Bain *et al.* (1978) and also by Eberhard in his Ph.D. dissertation (1976), but not in his subsequent article (1979). Also deserving comment are the longitudinal grooves of *O. lienalis*, described by Bain *et al.* (1978). Other characteristics such as ridges and internal striae of the cosmopolitan species have been described earlier by several authors (Beaver *et al.*, 1974; Bain, 1975; Bain *et al.*, 1978; Eberhard, 1976, 1979).

CUTICLE SPECIALIZATION OF *O. VOLVULUS*

The fourth-stage female has a thin cuticle with external striae like those normally seen in nematodes. Two young-adult females had a thin cuticle, 7-8 μm at mid-body, consisting of only one layer; the morphology of their cuticle depends on the degree of development of these worms. The smaller female, 22 mm long and 75 μm wide, had an externally striated cuticle, without any ridge, identical to that of the male (Bain, 1975). The female that was 35.6 mm long and 70 μm wide had delicate ridges, 4-6 μm apart, in the midpart of the body; internal striae were absent (Fig. 2H). The larger female seemed to be beginning to develop its cuticular specialization. In the fully developed female, the cuticle is 10-30 μm thick and is divided into two layers, the external layer with ridges 30-40 μm apart, and the internal layer with two striae between adjoining pairs of ridges (Beaver *et al.*, 1974; Bain, 1975). However, the anterior region of the female has the unspecialized morphology of all other *Onchocerca* species.

DISCUSSION

The specimen reported here was a female of an *Onchocerca* species characterized by the small diameter of the intestine, thin hypodermis (Beaver *et al.*, 1989), and presence of several regularly spaced transverse ridges. The transverse section appears to be from the posterior region because it passed through the ovaries. The cuticle was thinner (4-7 μm) than in other *Onchocerca* species, suggesting that the specimen was a young female. Observations of *O. volvulus* have shown cuticular thickening and acquisition of ridges during the adult stage. The young specimen of the human case reported here had salient ridges. Transverse ridges are lacking in *O. suzukii* and delicate in *O. lienalis*, *O. skrjabini*, and an *Onchocerca* sp. in Oita (Table II). Two other species, *O. gutturosa* and *O. d. japonica*, have salient ridges. In *O. gutturosa*, the cuticle is thickened in lateral areas, and the posterior region has ridges 10 μm apart, instead of the 40-80 μm for the specimen reported here. Moreover, in *O. gutturosa*, the body is dorsoventrally asymmetric, and the lateral chords are more towards the dorsal aspect, a difference accentuated in the posterior region (Bain *et al.*, 1978; Eberhard, 1976). This difference was not found here, but the validity of this characteristic for identifying a young individual is uncertain, because the degree of asymmetry may change during maturation. The morphology of the nematode found in the case reported here fits with that of *O. d. japonica*, in which species cuticular ridges are present, and sharp in the posterior region, with a distance between them of 50-80 μm (Table II). Therefore, this filaria is identified as *O. d. japonica*. Beaver *et al.* (1989) noted the great variation in the cuticle thickness of their specimens and concluded that the variation arose because of the thick transverse ridges. The symmetry of the body of the female worm and the other characteristics fit those of *O. d. japonica* (Table I), so *O. d. japonica* was also the causative agent of this first case from Oita.

Four of the ten cases of zoonotic onchocerciasis reported worldwide at this date have occurred in Oita, Japan, and the causative agent incriminated in at least three of these human cases is *O. d. japonica*, a parasite of wild boar. In other countries, *Onchocerca* species from cattle or horses have been implicated in human infections. The presence of *O. d. japonica* in wild boar has recently been reported (Uni *et al.*, 2001) and its prevalence is high in Oita: between August 22, 2001 and March 6, 2003, of the 24 wild boar examined for microfilariae in skin snips, 22 (92 %) had microfilariae of *O. d. japonica*. In contrast the prevalence of *O. gutturosa*, the other species with pronounced cuticular ridges, is 3.0 % (4/134) in cattle (Takaoka, 1997). The vectors of *O. gutturosa* and *O. lienalis* from cattle

Fig. 2. – Cuticular female characteristics of *Onchocerca* species from cattle and wild ungulates in Oita, and from young *O. volvulus*. A: Transverse ridges and internal striae, *O. gutturosa*, midbody (from Bain, 1975). B: External striae, *O. suzukii*, midbody (from Yagi *et al.*, 1994). C: Longitudinal grooves, *O. lienalis*, posterior region (from Bain *et al.*, 1978). D: Dorsoventral asymmetry, lateral thickenings, *O. gutturosa*, posterior region (from Bain *et al.*, 1978). E: Ridge, height (H) and width (W). F & G: Habitus and ridges of posterior region of *O. gutturosa* (from Bain, 1975) and *O. d. japonica* (from Uni *et al.*, 2001). H: Tiny cuticular ridges of *O. volvulus*, young-adult female 35.6 mm long and 70 μ m wide, at oesophageal-intestinal junction (* indicates the part with drawn ridges). I: Drawing of a transverse section of *Onchocerca* sp. of the present human case; ridges are sectioned at two places making the cuticle thicker (same as in Fig 1B).

	<i>O. suzukii</i>	<i>O. lienalis</i>	<i>O. gutturosa</i>	<i>O. skrjabini</i>	<i>O. dewittei japonica</i>	<i>Onchocerca</i> sp.
Body transverse ridges	Absent	Present	Present	Present	Present	Present
Longitudinal grooves	One lateral	All over the cuticle	Absent	Absent	Absent	Absent
Dorsoventral symmetry	Yes	Yes	Dorsal reduction	Yes	Yes	Yes
Cuticle laterally thickened	Slightly	No	Yes	Yes	No	No
Internal transverse striae	Absent	2 or undulations	4-8	3-4	Absent	2
Cuticle thickness med./lat.*	20-30/20-30	12-17/12-17	25**[29]-35/50	35/50	20-30/20-30	30/30
Outer <i>vs.</i> inner layer thickness	4:1	1:4	1:1	1:1 to 1:4	1:1	1:1
Ridge shape	—	Very sinuous	Straight to sinuous	Slightly sinuous	Straight	Slightly sinuous
Ridge section	—	Flattened	Sharp	Delicate	Sharp	Delicate
Ridge height/width*	—	[3/22]	12/5-10	6/12	18-25/25-50	3/8
Inter-ridge space*	—	25-45	70-80	48-55	140*-250	25-40
Lateral chord height/width*	37/155	12-20/55-65	20/100	15/50	20/60	10/38
Muscle height*	52	25	40	15-20	50-60	23
Muscle number per quadrant	5-7	5-7	4-6	5-7	6-7	—
Intestine*	20-48	13-30	20-37	38	17	15
Diameter at midbody*	228-430	[150] 180-220	140-225	170-330 [340]	190-260	60-120
Diameter at posterior part*,***	170	160-190	75-90	150	150	130
Diameter at anus*	150	85	70-95	110	70-120	60
Cuticle thickness med./lat.*	30-50/30-50	20/20	14/33	16/16	20-25/20-25	—
Ridge height/width*	—	External striae	10/10	External striae	10-20/22-30	External striae
Inter-ridge space*	—	—	10	—	50-80	—
Posterior part habitus	Straight	Tortuous	Spirally coiled	Straight	Straight	Spirally coiled
Host(s) in Oita	<i>Capricornis crispus</i> Cattle	Cattle	Cattle	<i>Cervus n. nippon</i>	<i>Sus scrofa leucomystax</i>	<i>Cervus n. nippon</i>
References	Yagi <i>et al.</i> , 1994 Uni <i>et al.</i> , 1998	Bain <i>et al.</i> , 1978 Eberhard, 1979	Bain, 1975 Bain <i>et al.</i> , 1978 Eberhard, 1979	Bain & Schulz-Key, 1974 Yagi <i>et al.</i> , 1994 Uni (unpublished)	Uni <i>et al.</i> , 2001 Uni (unpublished)	Ongoing study Uni (unpublished)

* Measurements in micrometers. ** Young female. *** About 250 µm anterior to anus. Med., median; lat., lateral. [] From Eberhard, 1979, or Yagi *et al.*, 1994 according to the species.

Table II. – *Onchocerca* species found in Oita: characteristics useful for diagnosis from histologic sections of human nodules.

in Oita have been identified as being black flies (Takaoka *et al.*, 1989; Takaoka & Bain, 1990; Takaoka, 1994, 1999). The yet unidentified vectors of *O. d. japonica* from wild boar must be anthrozoophilic.

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