INFECTIVE LARVAE OF FIVE *ONCHOCERCA* SPECIES FROM EXPERIMENTALLY INFECTED *SIMULIUM* SPECIES IN AN AREA OF ZOONOTIC ONCHOCERCIASIS IN JAPAN

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Summary:

Microfilariae of five *Onchocerca* species, *O. dewittei japonica* (the causative agent of zoonotic onchocerciasis in Oita, Kyushu, Japan) from wild boar (*Sus scrofa*), *O. skrjabini* and *O. eberhardi* from sika deer (*Cervus nippon*), *O. lienalis* from cattle, and an as yet unnamed *Onchocerca* sp. from wild boar, were injected intrathoracically into newly-emerged black flies of several species from Oita to search the potential vector(s) of these parasites and identify their infective larvae. Development of *O. dewittei japonica* microfilariae to the infective larvae occurred in *Simulium aokii*, *S. arakawae, S. bidentatum, S. japonicum, S. quinquestriatum*, and *S. rufibasis* while development of infective larvae of *O. skrjabini, O. eberhardi*, and the unnamed *Onchocerca* sp. was observed in *S. aokii, S. arakawae*, and *S. bidentatum*. Development of *O. lienalis* microfilaria to infective larvae occurred in *S. arakawae*. Based on the morphology of infective larvae obtained, we proposed a key of identification of *Onchocerca* infective larvae found in Oita. We also reconsider the identification of three types of infective larvae previously recovered from *Simulium* species captured at cattle sheds: the large type I larvae that may be an undescribed species; the small type III identified as *O. lienalis* may include *O. skrjabini* too; the intermediary type II that may be *O. gutturosa*, or *O. dewittei japonica*, or the unnamed *Onchocerca* sp. of wild boar.

KEY WORDS : *Onchocerca*, experimental infection, infective larvae, *Simulium*, vector, zoonosis, Japan.

Résumé : LARVES INFECTANTES D'ONCHOCERQUES OBTENUES EXPÉRIMENTALEMENT CHEZ DES SIMULIES, DANS UNE RÉGION D'ONCHOCERCOSE ZOONOTIQUE AU JAPON

*Afin d'identifier les vecteurs potentiels et les larves infectantes des onchocerques à Oita, Kyushu, Japon, les jeunes imagos de plusieurs espèces de simulies ont été inoculés intrathoraciquement avec les microfilaires de cinq espèces d'*Onchocerca : O. dewittei japonica, *parasite du sanglier* Sus scrofa *et agent de l'onchocercose zoonotique locale ;* O. skrjabini *et* O. eberhardi *du cerf sika* Cervus nippon *;* O. lienalis *du bétail ; une autre onchocerque du sanglier, non encore nommée,* Onchocerca *sp.* Onchocerca dewittei japonica *s'est développé jusqu'au stade infectant chez* Simulium aokii, S. arakawae, S. bidentatum, S. japonicum, S. quinquestriatum, *et* S. rufibasis *;* O. skrjabini, O. eberhardi *et* Onchocerca *sp. chez* S. aokii, S. arakawae *et* S. bidentatum *;* O. lienalis *chez* S. arakawae. *Nous proposons une clé d'identification des larves infectantes basée sur la morphologie. Nous reconsidérons l'identification des trois types de larves précédemment trouvées chez des simulies capturées aux abris du bétail. Le grand type I semble être une filaire inconnue ; le petit type III,* O. lienalis *ou* O. skrjabini *; le type II de taille intermédiaire,* O. gutturosa *(mais cette espèce est en général transmise par* Culicoides*), ou* O. d. japonica, *ou* Onchocerca *sp. du sanglier.*

MOTS CLÉS : Onchocerca, *infection expérimentale, larves infectantes,* Simulium, *vecteur, zoonose, Japon.*

INTRODUCTION

Zoonotic onchocerciasis is rare in humans with 13 cases reported in the world during the past forty years, but five of them are described from Oita, Kyushu Island, Japan (Beaver *et al*., 1989; Hashimoto *et al*., 1990; Takaoka *et al*., 1996, 2001, 2004, 2005). The causative agent was not a parasite of cattle or horse, as elsewhere, but *Onchocerca dewittei japonica* Uni, Bain & Takaoka, 2001, a common filaria parasite of wild boar (Takaoka *et al*., 2001; Uni *et al*., 2001). To investigate the transmission and the background of this zoonotic onchocerciasis, we have examined filariae from wild ungulates in Japan, the wild boar *Sus scrofa* Linnaeus, 1758, the sika deer *Cervus nippon* Temminck, 1838, the serow *Naemorhedus crispus* (Temminck, 1845), as well as cattle (*Bos taurus*), and the putative vectors, the black flies (Diptera: Simuliidae). These studies showed a great diversity among the genus *Onchocerca* as well as other Onchocercinae (Takaoka *et al*., 1989; Takaoka, 1990, 1994; Takaoka & Bain, 1990; Yagi *et al*., 1994; Uni *et al*., 2001, 2002, 2004, 2006, 2007). The picture of the filarial fauna is however still incomplete, and a few kinds of microfilariae, without corresponding adult worms, remain unidentified.

In Oita, *Onchocerca* species so far reported are *O. dewittei japonica* in 92 % of wild boar (Takaoka *et al*., 2004);

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O. skrjabini Rukhlyadev, 1964 and *O. eberhardi* Uni & Bain, 2007 in 36 % and 81 % of sika deer, respectively (Uni *et al.*, 2007). In addition, *Onchocerca*-like microfilariae larger than those of *O. dewittei japonica* were observed in 44 % of wild boar during this study and provisionally referred to *Onchocerca* sp. Adult worms found in a Japanese serow, in Oita, identified to *O. suzukii* Yagi, Bain & Shoho, 1994 (cited in Takaoka *et al.*, 2004), were reidentified to *Loxodontofilaria caprini* Uni & Bain, 2006 (Uni *et al.*, 2006). The vectors of these four species are not known, except for *O. skrjabini*, which is transmitted by two *Simulium* species in Europe (Schulz-Key & Wenk, 1981).

From cattle three species are known, of which two worldwide species, *O. gutturosa* Neumann, 1910 and *O. lienalis* (Stiles, 1982), were found in 3.0 % and 17.2 % of local cattle, respectively (Takaoka, 1997). It was shown that ceratopogonids are the main vectors of *O. gutturosa*, in Europe (Bain, 1979; Dohnal *et al.*, 1990) and in Africa (El-Sinnary & Hussein, 1980), whereas simuliids are responsible for transmission of *O. lienalis* (Lok *et al.*, 1983; Dohnal *et al.*, 1990). However there is one report of *O. gutturosa* development in a *Simulium* species, in Tanzania (Mwaiko, 1981). Infective larvae (third-stage larvae) of *O. lienalis* are shorter than those of *O. gutturosa* (Bain & Chabaud, 1986). A third species, tentatively identified as *O. suzukii* on the basis of the characteristic coiled posterior region of the microfilariae (Uni *et al.*, 1998), was found in 6.7 % of cattle (Takaoka, 1997), and reported to be transmitted by *S. bidentatum* (Shiraki, 1935) (Takaoka, 1994).

We previously described three types of infective *Onchocerca* larvae from female black flies collected from a cattle shed in Oita. These were designated type I, II, and III and identified as *O.* sp., *?O. gutturosa*, and *?O. lienalis*, respectively (Takaoka & Bain, 1990). Type I was shown to be identical to the third filarial species in cattle. In this study we carried out experimental infections of adult black flies with microfilariae of *O. dewittei japonica*, *O. skrjabini*, *O. eberhardi*, the unnamed *Onchocerca* sp. of wild boar, and *O. lienalis*, and determined the ability of simuliids to support development of infective larvae. The morphological characteristics of infective larvae recovered from experimental infections were compared with those of infective larvae previously described in Oita (Takaoka, 1990, 1994; Takaoka & Bain, 1990), and the criteria for identification of the infective larvae found in local black flies were reevaluated.

MATERIALS AND METHODS

Experimental infections were done by injecting black flies with microfilariae, following the method used by Reid (1979).

Adult black flies were reared from the pupae collected at Hasama-machi and Oita City, Oita Prefecture, Kyushu Island, Japan. They were morphologically identified to species using the established keys (Takaoka, 1977). Wild boar and deer captured in Oita Prefecture were obtained from a licensed hunter. Cattle were obtained from an abattoir in Oita. Microfilariae were isolated from skin snips of the ears and the neck of wild boar for *O. dewittei japonica* and the unnamed *Onchocerca* sp., the ears of wild sika deer for *O. skrjabini* and *O. eberhardi*, and the abdomen of cattle for *O. lienalis*. The microfilariae were harvested into medium 199 (Sigma, MO, USA) containing penicillin (200 U/ml), streptomycin (200 µg/ml), and foetal bovine serum (20 %). Identification of microfilariae to species followed Uni *et al.* (2001) for *O. dewittei japonica*, Bain & Schulz-Key (1974) and Yagi *et al.* (1994) for *O. skrjabini*, Uni *et al.* (2007) for *O. eberhardi*, and Bain *et al.* (1978) for *O. lienalis*. Microfilariae of the unnamed *Onchocerca* sp. were easily distinguished from those of *O. dewittei japonica* by their body lengths (210-246 µm for *O. dewittei japonica* (n = 7) *vs* 280-331 µm for *Onchocerca* sp. (n = 5)). Newly-emerged black flies were inoculated intrathoracically with two to eight microfilariae of each species in 199 medium (see above). The black flies were housed individually and fed on 30 % sucrose solution at 25° C for 10 days. Surviving flies were dissected in 0.9 % saline solution and examined for filarial larvae. Larvae recovered were fixed in 4 % formalin solution and treated with lactophenol solution for morphometric observations. Generic diagnosis of infective larvae followed Bain & Chabaud (1986).

RESULTS

Experimental infections of black flies and filarial development

Tables I and II show the results of experimental infections of *Simulium* species with two species of *Onchocerca* from boar. Females of six *Simulium* species were positive for infective larvae of *O. dewittei japonica*. The larvae in *S. aokii* (Takahasi, 1941), *S. arakawae* Matsumura, 1915, *S. bidentatum*, and *S. rufibasis* Brunetti, 1911 were recovered from the head, thorax, and abdomen. The larvae in *S. japonicum* Matsumura, 1931 and *S. quinquestriatum* (Shiraki, 1935) were recovered from the head and thorax, and the head, respectively. No larva was found in *S. uchidai* (Takahasi, 1950). Males of two species had infective larvae of *O. dewittei japonica*. The larvae were found in the head and abdomen of *S. aokii* and the thorax of *S. arakawae*. One second-stage larva and one first-stage larva were each found in the thorax of *S. bidentatum* and *S. aokii*. Females of three *Simulium* species were

***Simulium* spp.**	**Sex of flies**	**No. flies inoculated**	**No. flies dissected**	**No. flies with IL (%)**	**No. IL**
S. aokii	F	25	21	4 (19)	7
	M	8	4	1 (25)	2
S. arakawae	F	26	14	10 (71)	17
	M	3	1	1 (100)	1
S. bidentatum	F	21	11	6 (55)	9
	M	6	3	0 (0)	0
S. japonicum	F	1	1	1 (100)	2
S. quinquestriatum	F	2	1	1 (100)	1
	M	1	0	– (–)	–
S. rufibasis	F	9	4	3 (75)	4
	M	3	0	– (–)	–
S. uchidai	F	2	0	– (–)	–
Total	F	86	52	25	40
	M	21	8	2	3

F, female(s); M, male(s); IL, infective larva(e); –, relative data not obtainable.

Table I. – Results of experimental infections of *Simulium* species with *Onchocerca dewittei japonica*.

***Simulium* spp.**	**Sex of flies**	**No. flies inoculated**	**No. flies dissected**	**No. flies with IL (%)**	**No. IL**
S. aokii	F	12	5	5 (100)	6
	M	4	2	0 (0)	0
S. arakawae	F	5	3	2 (67)	3
	M	2	0	– (–)	–
S. bidentatum	F	14	10	4 (40)	5
	M	8	1	0 (0)	0
S. rufibasis	F	4	3	0 (0)	0
	M	3	2	0 (0)	0
S. uchidai	F	3	3	0 (0)	0
Total	F	38	24	11	14
	M	17	5	0	0

F, female(s); M, male(s); IL, infective larva(e); –, relative data not obtainable.

Table II. – Results of experimental infections of *Simulium* species with *Onchocerca* sp. from wild boar.

positive for infective larvae of *Onchocerca* sp. The larvae were found in the head and thorax of *S. aokii*, the head of *S. arakawae*, and the thorax and abdomen of *S. bidentatum*. No infective larva of *Onchocerca* sp. was found in any species of male black fly. Two microfilariae were found in the head and abdomen of one *S. rufibasis*.

Three *Simulium* species were positive for infective larvae of *O. skrjabini* from deer (Table III). The larvae were found in the head of *S. aokii*, the head and abdomen of *S. arakawae*, and the head and abdomen of *S. bidentatum*. One first-stage larva and one microfilaria were found together in the thorax of *S. bidentatum*.

Three *Simulium* species had infective larvae of *O. eberhardi* from deer (Table IV), which were found in the head of *S. aokii* and *S. bidentatum*, and the head and abdomen of *S. arakawae*.

***Simulium* spp.**	**No. flies inoculated**	**No. flies dissected**	**No. flies with IL (%)**	**No. IL**
S. aokii	31	12	4 (33)	6
S. arakawae	35	19	6 (32)	8
S. bidentatum	17	11	3 (27)	3
S. japonicum	1	0	– (–)	–
S. uchidai	8	3	0 (0)	0
Total	92	45	13	17

IL, infective larva(e); –, relative data not obtainable.

Table III. – Results of experimental infections of female *Simulium* species with *Onchocerca skrjabini*.

***Simulium* spp.**	**No. flies inoculated**	**No. flies dissected**	**No. flies with IL (%)**	**No. IL**
S. aokii	18	12	2 (17)	2
S. arakawae	14	14	8 (57)	13
S. bidentatum	10	10	3 (30)	3
S. uchidai	2	1	0 (0)	0
Total	44	37	13	18

IL, infective larva(e).

Table IV. – Results of experimental infections of female *Simulium* species with *Onchocerca eberhardi*.

Simulium spp.	No. flies inoculated	No. flies dissected	No. flies with IL (%)	No. IL
S. aokii	1	0	– (–)	–
S. arakawae	5	2	1 (50)	2
S. bidentatum	7	3	0 (0)	0
Total	13	5	1	2

IL, infective larva(e); –, relative data not obtainable.

Table V. – Results of experimental infections of female *Simulium* species with *Onchocerca lienalis*.

Out of three *Simulium* species injected with *O. lienalis* from cattle, *S. arakawae* supported the complete development (Table V). Two infective larvae were found in the head of the fly.

Description of infective larvae

The infective larvae of the five *Onchocerca* species had a trapezoidal head, a short buccal capsule (≤ 5 µm); four tiny external labial papillae seen in a few specimens, four salient cephalic papillae; amphids, rarely identified. A oesophagus with thin posterior glandular part was evident but its junction with intestine was often unclear. In some female larvae, the genital primordium was observable at level of the glandular oesophagus.

Onchocerca species from wild boar (Figs 1 and 2, Table VI)

Onchocerca dewittei japonica (Fig. 1A-K). Six specimens were studied: three from *S. aokii* abdomen, head, and thorax, and three from *S. arakawae* thorax. Habitus: attenuated anterior fourth of body (Fig. 2O); caudal end slightly attenuated; posterior region straight, or with slight dorsal preanal bent. Tail: straight, or bent dorsally, rarely ventrally; cylindrical or slightly constricted at mid-length. Tail extremity: cuticle thickened; conical, or round with a tiny axial protuberance which is round in ventral view (Fig. 1H) and truncated in lateral view (Fig. 1I); lateral lappets identified in two/six specimens. The unnamed *Onchocerca* sp. (Fig. 1L-Q). Three specimens were studied: two recovered from *S. aokii* thorax and one from *S. arakawae* head. Habitus: anterior fourth of body attenuated; no preanal bent or very slightly and directed dorsally. Tail: straight, cylindrical. Tail extremity: cuticle thickened; round, smooth or corrugated.

Onchocerca species from sika deer (Fig. 2, Table VI)

Onchocerca skrjabini (Fig. 2A-F). Six specimens were studied: three recovered from the heads of *S. aokii,* and three from *S. arakawae*. These were short larvae (Table VI). Habitus: attenuated anterior third of body, posterior part less attenuated and shortly; a slight dorsal bent just anterior to anus. Tail: almost cylindrical, or hardly constricted at mid-length, or attenuated to end. Tail extremity: thickened cuticle; round, or slightly conical, rarely an axial smooth point; caudal lappets extremely tiny, identified in specimens from *S. aokii* only. *Onchocerca eberhardi* (Fig. 2G-M). Eight specimens were studied (all recovered from head): one from *S. aokii*, one from *S. bidentatum*, and others from *S. arakawae*. Habitus: slightly and similarly attenuated at both ends; usually slightly bent dorsally anterior to anus, straight in other specimens. Tail: cylindrical, or slightly constricted at mid-length, or slightly attenuated. Tail extremity: thickened cuticle; slightly conical; corrugated or smooth surface.

Onchocerca species from cattle (Figs 1 and 2, Table VI)

Onchocerca lienalis (Fig. 1R & S). Two specimens were recovered from the head of *S. arakawae*. Habitus: slightly attenuated at both extremities (Fig. 2P); body relatively thick; slight dorsal preanal bent. Tail: cylindrical or attenuated (Fig. 2S). Tail extremity: attenuated, round.

DISCUSSION

Onchocerca spp. development in *Simulium* species from Oita

It has been shown for the first time that six blackfly species, *S. aokii, S. arakawae, S. bidentatum, S. japonicum, S. quinquestriatum*, and *S. rufibasis*, all common, are able to support the larval develop-

	O. d. japonica	*Onchocerca* sp.	*O. skrjabini*	*O. eberhardi*	*O. lienalis*
Body length (BL)*	880-1,030	780-940	510-590	380-425	465-473
Body width (BW)*	18-22	22	14-16	14-15.5	16.5-18
Oesophagus length*	472-685	430-700	335-380	210-240	290-305
Tail length (TL)*	45-55	48-52	36-40	26-34	31-32
Tail width at anus (TW)*	13-18	14-19	11.5-14	12-13	16-17
BW × 100/BL: mean (extremes)	2.0 (1.9-2.2)	2.4 (2.3-2.6)	2.6 (2.4-3.1)	3.6 (3.4-4.0)	3.4-3.8
TL × 100/BL: mean (extremes)	5.2 (4.8-6.1)	5.8 (5.5-6.1)	6.7 (6.4-7.0)	7.2 (6.8-8.0)	6.5-6.8
Tail ratio TL/TW: mean (extremes)	3.0 (2.6-3.6)	3.0 (2.7-3.4)	2.9 (2.5-3.3)	2.3 (2.0-2.6)	1.8-1.9
No. IL (No. oeso.) measured	6 (5)	3 (3)	6 (2)	8 (4)	2 (2)

* Measurements in µm. IL, infective larvae.

Table VI. – Infective larvae of *Onchocerca* spp. obtained from *Simulium* spp. injected with microfilariae in Oita, Japan.

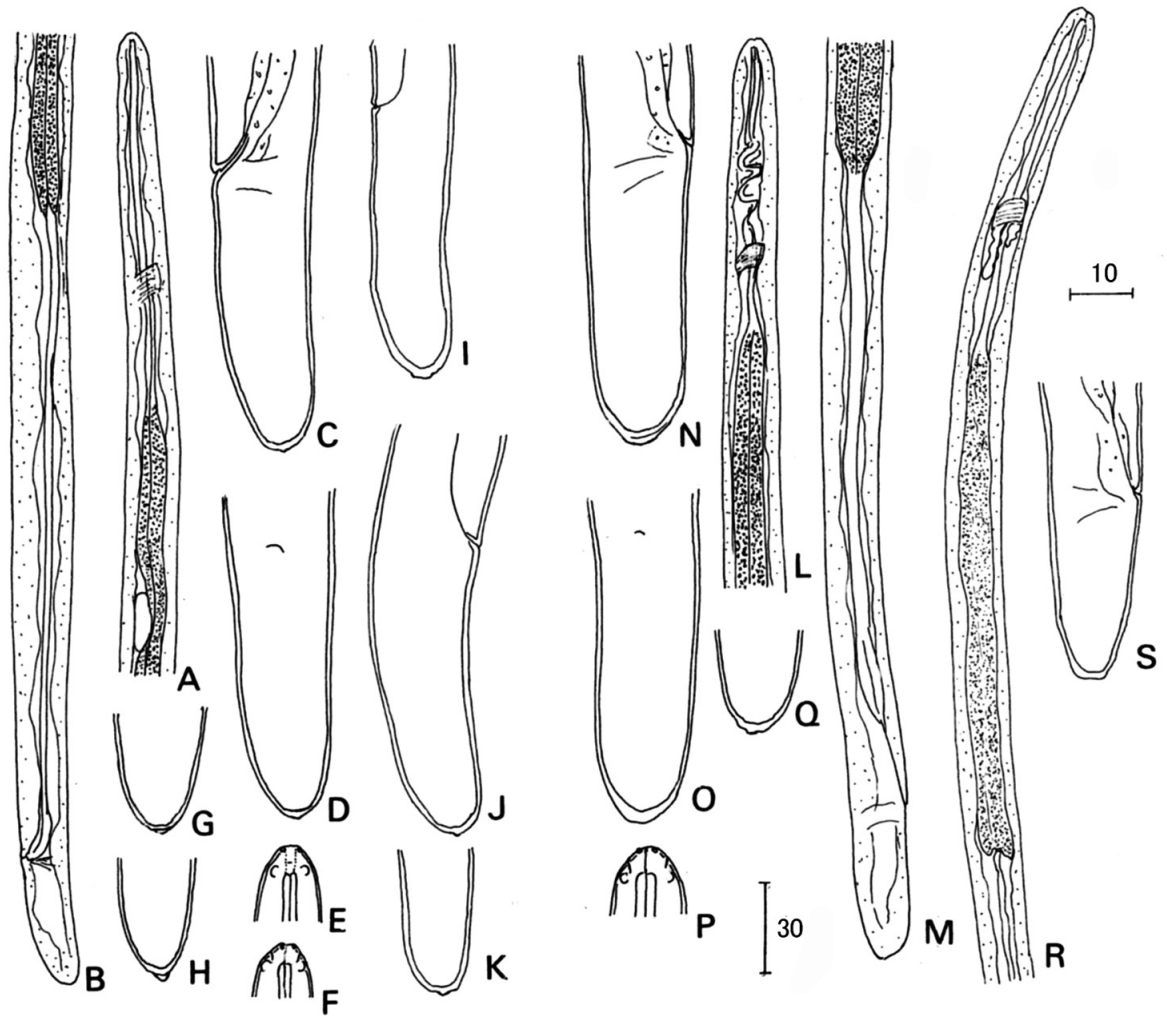

Fig. 1. – Infective larvae of *Onchocerca dewittei japonica* and *Onchocerca* sp. from wild boar, and *O. lienalis* from cattle in Oita. A-K. *O. dewittei japonica*. A. Anterior region, left lateral view. B. Posterior region, left lateral view. C. Tail, left lateral view. D. Tail, ventral view. E & F. Head, lateral and median view, respectively. G & H. Tail extremities, ventral view. I. Tail, left lateral view. J. Tail, right lateral view. K. Tail extremity, ventral view. L-Q. *Onchocerca* sp. L. Anterior region, left lateral view. M. Posterior region, right lateral view. N. Tail, right lateral view. O. Tail, ventral view. P. Head, median view. Q. Tail extremity, ventral view. R-S. *O. lienalis*. R. Anterior region, left lateral view. S. Tail, right lateral view. Scales in µm: A, B, L, M, R, 30. Others, 10.

ment of *O. dewittei japonica*, the causative agent of zoonotic onchocerciasis in Oita, Japan. The infection rate was high in all species examined.

Microfilariae of the unnamed *Onchocerca* sp. also successfully developed to infective larvae in *S. aokii*, *S. arakawae*, *S. bidentatum*, but only in female flies, unlike *O. dewittei japonica*. However, the significance of this observation remains to be determined as only a very few flies were investigated.

The infective larvae of *O. dewittei japonica* and the unnamed *Onchocerca* sp. from wild boar obtained in this experiment resemble those of type II *Onchocerca* found in *S. bidentatum* (Table VII). These were tentatively assumed to be *O. gutturosa* of cattle, by Takaoka & Bain (1990), before the discovery of wild boar parasites.

Onchocerca skrjabini and *O. eberhardi* successfully larval developed in *S. aokii*, *S. arakawae*, and *S. bidentatum*. These three black-fly species are possible natural vectors of four *Onhcocerca* species, the two *Onchocerca* species from sika deer in Japan, and the two species from wild boar, *O. dewittei japonica* and *Onchocerca* sp.

The present study shows that *O. lienalis* develops in *S. arakawae*. The two infective larvae obtained are similar to that found by Takaoka (1990) following injection of microfilariae from cattle in *S. arakawae,* and to the type III from wild-caught *S. arakawae* (Takaoka & Bain, 1990). Thus *S. arakawae* is also a vector of *O. lienalis*.

No infective larvae resembling type I (Takaoka, 1990, 1994; Takaoka & Bain, 1990) were obtained with the *Onchocerca* microfilariae injected in this study.

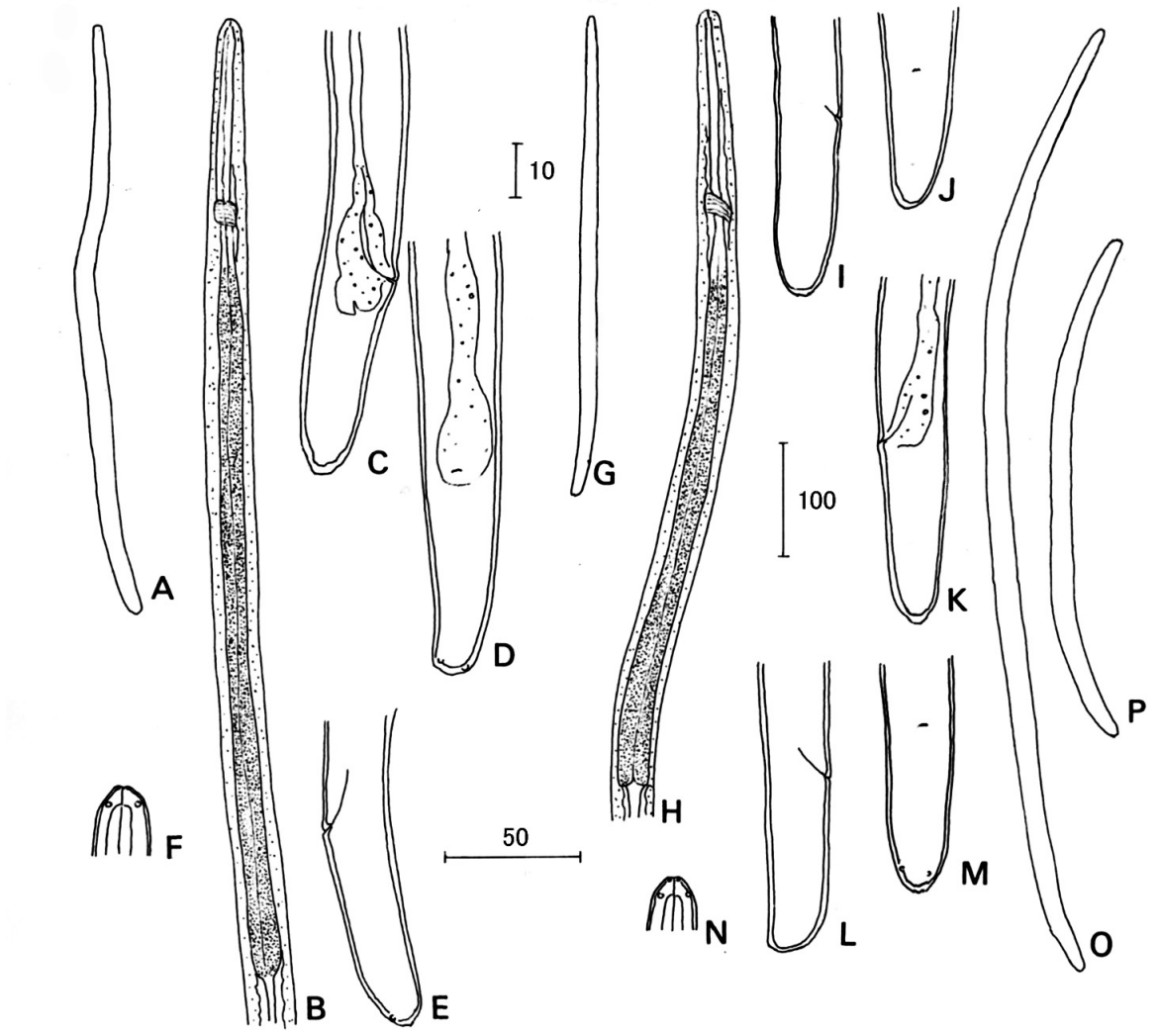

Fig. 2. – Infective larvae of *Onchocerca* species from sika deer, wild boar and cattle from Oita. A-F. *O. skrjabini*. A. Habitus, left lateral view. B. Anterior region, ventral view. C. Tail, right lateral view. D. Tail, ventral view. E. Tail, left lateral view. F. Head, median view. G-M. *O. eberhardi*. G. Habitus, right lateral view. H. Anterior region, right lateral view. I. Tail, right lateral view. J. Tail, ventral view. K. Caudal region, left lateral view. L & M. Tail, right lateral and ventral view, respectively. N. Head, median view. O. *O. dewittei japonica*, habitus, left lateral view. P. *O. lienalis*, habitus, left lateral view. Scales in µm: A, G, O, P, 100. B, H, 50. Others, 10.

Diagnosis of infective larvae of the *Onchocerca* species

The five species of *Onchocerca* studied morphologically resemble one another and were typical of the genus (Bain & Chabaud, 1986). The caudal morphology, particularly at the extremity, showed slight variations within species. Unlike *Onchocerca* species from African suids, the two species recovered from wild boar lacked a characteristic caudal point (Wahl & Bain, 1995). Other morphometric characters were more constant (Table VI). The infective larvae of *O. dewittei japonica* and the unnamed *Onchocerca* sp. are the longest (780-1,030 µm), those of *O. eberhardi* are the shortest (380-425 µm) and those of *O. lienalis* and *O. skrjabini* are intermediate (465-473 µm and 510-590 µm, respectively). It should be noted however that the dimensions recorded here for larvae of *O. lienalis* are short compared to those reported by Supperer (1952) and Steward (1937) at 540-563 µm and 650 µm, respectively (Table VII).

There is also an important morphometric comparison to be made with respect to the relative dimensions of the body and tails of the parasites. For example *O. dewittei japonica* and *Onchocerca* sp. from wild boar have the smallest tail-body ratios of between 4.8-6.1; *O. eberhardi* and *O. lienalis* have the greatest body width-length ratios at 3.4-4.0 and 3.4-3.8, respectively. The habitus is very likely another valuable specific character; indeed *O. skrjabini* is distinct to *O. lienalis* with the more attenuated anterior part, but this character will have to be confirmed with more specimens.

	O. lienalis[a]	*O. gutturosa*[b]	Type I[c]	Type III[c]	Type I[d]	Type II[d]	Type III[d]
Geographical site	**Europe**		**Oita**				
Body length (BL)*	540-563[e]	770-900	1,102-1,231	478	1,037-1,407	870-950	450-530
Body width (BW)*	19-20	17-19	25.8-26.6	18.8	24-26.4	18.9-21	16.6-18
Oesophagus length*	324	470-572	494-600	304	500-650	520-610	330-350
Tail length (TL)*	40	38-47	40.0-41.8	30.1	38-45	36-51	32-37
Tail width at anus (TW)*	–	14.6-16.2	–	–	–	–	–
BW × 100/BL	–	1.9-2.4	2.1-2.3	3.9	1.8-2.4	2.0-2.3	3.3–3.6
TL × 100/BL	–	4.6-5.4	3.3-3.6	6.2	2.8-3.6	4.0-5.8	6.0-7.2
Tail ratio TL/TW	–	2.5-2.9	1.9-2.2	2.0	1.7-2.2	2.4-2.8	2.1
No. IL (No. oeso.) measured	–	4 (4)	7 (7)	1 (1)	10 (7)	6 (4)	5 (4)
Suspected species by the present study			Undescribed species	*O. lienalis*	Undescribed species	? *O. gutturosa*, *Onchocerca* species from wild boar	*O. lienalis*, ? *O. skrjabini*

IL, infective larvae; –, not available. *Measurements in µm. [a]IL from *Simulium*; Supperer, 1952. [b]IL from *Culicoides nubeculosus*; Bain, 1979. [c]IL from *Simulium* spp. infected with microfiraliae taken from cattle skin or the stomach of newly-blood fed *Simulium* captured at cattle shed; Takaoka, 1990. [d]IL from wild-caught *Simulium* spp. at cattle shed; Takaoka & Bain, 1990; Takaoka, 1994. [e]650 µm in Steward, 1937.

Table VII. – Infective larvae of *Onchocerca lienalis* and *O. gutturosa* in Europe and those of filarial species from *Simulium* spp. in Oita, Japan.

Specific identification remains difficult mainly because the morphological characters of two worldwide species, *O. gutturosa* and *O. lienalis*, are not sufficiently established. However our experimental infections provide a valuable base for identifying the infective larvae from wild-caught *Simulium* spp.

DIAGNOSIS OF FILARIAL LARVAE FROM WILD-CAUGHT *SIMULIUM* SPP. IN OITA

An identification key is proposed on the basis of the present study (Table VI), and published data (Table VII) concerning *O. lienalis* and *O. gutturosa* from experimental cycles in simuliids and *Culicoides* in Europe (Supperer, 1952; Bain, 1979), and the infective larvae obtained from injected microfilariae from cattle (Takaoka, 1990) and those found in wild black flies collected at cattle shed in Oita (Takaoka & Bain, 1990; Takoaka, 1994).

From the present descriptions of five *Onchocerca* species in Oita identification of the three types of infective larvae is amended. Type III is *O. lienalis* or *O. skrjabini*. Type II is either *O. dewittei japonica*, or the unnamed *Onchocerca* sp. from wild boar, or *O. gutturosa*. Type I infective larvae differ from the *Onchocerca* species of this study, and from other *Onchocerca* species (Bain & Chabaud, 1986).

Type I larvae are thick, long, and with a small tail to body ratio. It had been suggested that they were infective larvae of *O. suzukii*, a parasite of the serow (Uni *et al.*, 1998). However this hypothesis should be revised in view of our recent description of *L. caprini* from Japanese serows (Uni *et al.*, 2006). This parasite resembled *O. suzukii* with its coiled microfilariae and it could not be initially recognized as a distinct species. *Loxodontofilaria caprini* microfilariae are found to be smaller (105-140/5-8 µm) and to possess a more coiled tail than those of *O. suzukii* (245-300/7.5 µm, unpublished data by Uni). On the other hand, the microfilariae with coiled tail recovered from the skin of cattle were 156-181 µm long by 4-5 µm wide (Uni *et al.*, 1998). It is suggested that the type I is neither *O. suzukii* nor *L. caprini* but an undescribed filaria, possibly close to these genera.

KEY OF IDENTIFICATION OF INFECTIVE LARVAE FROM *SIMULIUM* SPECIES IN OITA, JAPAN

1-(6) Body length ≤ 1,030 µm and body width ≤ 22 µm (14-22)
2-(3) Body length from 770 to 1,030 µm
Onchocerca dewittei japonica
O. sp. from wild boar
Type II
O. gutturosa
3-(2) Body length < 770 µm
4-(5) Body length from 450 to 600 µm
O. skrjabini
O. lienalis
Type III
5-(4) Body length < 450 µm (380-425)
O. eberhardi
6-(1) Body length > 1,030 µm (1,037-1,407) and body width ≥ 24 µm (24-26)
Type I

In conclusion several *Simulium* species are putative vectors of *O. dewittei japonica*, the agent of zoonotic

onchocerciasis in Oita. In the future, molecular analysis will contribute to the identification of infective larvae of *Onchocerca* species.

ACKNOWLEDGEMENTS

We are grateful to Dr Y. Otsuka and Ms C. Aoki, Oita University, and Ms H. Sato for their kind help. We thank Mr H. Goto and the staffs of Oita Prefectural Center for Meat Inspection for their kindness in collecting the animals. Our sincere thanks are due to Prof. David Taylor for his valuable comments and Dr Tarik Attout for his kind help on figures presentation.

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Reçu le 15 octobre 2007
Accepté le 21 décembre 2007