4. Review Articles Related to the Cooperation Project (Republications)

2) Onchocerciasis[*]

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Although onchocerciasis in cattle and horses in Japan had been noted in the veterinary field since the early 1950s, research on human onchocerciasis that is caused by *Onchocerca volvulus* was carried out only in the early 70s. It began when I did a brief study of skin test for onchocerciasis cases in Guatemala, Central America, in collaboration with Dr. H. Figueroa [1] using a *Dirofilaria* antigen [2]. Dr. Figueroa was an admirer of Dr. R. Robles, who discovered the presence and distribution of onchocerciasis on the American continents. Later on, many Japanese parasitologists participated in this field of research through the international health projects sponsored by Japan's ODA (Overseas Development Assistance) agency (Overseas Technical Cooperation Agency (OTCA)/ and later Japan International cooperation Agency (JICA)) that were implemented in Ethiopia (after1969), Guatemala (after 1975) and Nigeria (after 1983).

STUDIES ON HUMAN ONCHOCERCIASIS IN JAPAN

Studies on microfilaria

The skin snipping method is a basic technique for the diagnosis of onchocerciasis. This diagnostic method had not been standardized in the 1960's. Research on human onchocerciasis by Japanese scientists began with the investigation of the distribution of *O. volvulus* microfilaria in the skin of the patient after being snipped off. In a study in Ethiopia, it was found that more microfilariae could be recovered and detected from the snipped skin after being placed in warm saline if the skin were not macerated or teased, and the incubation time was prolonged to 6–7 hours [3, 4]. Later, a group of German researchers used collagenase to digest the snipped skin and showed that this is a better method to assess microfilarial density in the skin of the patient. By using the collagenase method to confirm the total number of microfilariae within a snipped skin, Fukumoto *et al.* [5] reported that only 70% of the microfilariae were released and thus could be detected from the

snipped skin after 1 hour incubation in saline. We examined the density fluctuation of microfilariae in the skin of 9 Guatemalan patients at every 4 h. for 20 h. using the quantitative skin snip method. Since there was no fluctuation in microfilaria density with passage of time, it was concluded that microfilarial periodicity, which is seen for the lymphatic filaria, could not be observed for onchocercal microfilariae [6]. Furthermore, it was also demonstrated that there was no difference in the microfilarial density between sun-exposed and masked skins. Hashiguchi *et al.* [7] reported similar finding when they found that the microfilarial density in 4 patients, despite showing a rise between 5 p.m. and 1 a.m., had no relation to the frequency of the blackfly bites, thus dispelling the existence of any microfilarial periodicity. However, they showed a seasonal rise in microfilarial density in 11 patients between August to October, which coincided with the peak biting period of the vector blackfly, *Simulium ochraceum* [8]. Kawabata *et al.* [9] showed that the microfilariae were found distributed most densely in the iliac crest among males and in the scapular region among the females. They also noted that there is a potential risk of eye lesions in people with heavy infections that showed high densities of microfilariae in the head and neck. In a study in Nigeria, Ufomadu *et al.* [10] reported that in 95% of the patients, the microfilarial density was highest in the iliac crest but among patients who also showed microfilariae in other body parts, no microfilaria could be detected from the iliac crest of 23.7% of them. With regard to the standardization of skin snipping method, Zea *et al.* [11] compared the efficiency of the lancet/knife, Walser type and the Holth type corneoscleral punches on 108 infected volunteers. They recommended the Holth type punch for its efficacy and safety in mass diagnosis.

Aoki *et al.* [12] inoculated *O. volvulus* microfilariae into the inguinal region of mice and monitored their migration in the animal host. They observed that the injected microfilariae accumulated in the tail and survived for at least 12 weeks. On the contrary, when the microfilariae were subcutaneously inoculated into the head, most of the mi-

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crofilariae accumulated in the ears. Shimada *et al.* [13] examined the effect of Diethyl carbamazine (DEC), a filaricidal agent, on the ingestion of microfilariae from infected person into the vector blackfly. Two onchocerciasis patients were each orally administered 100 mg of DEC and then several hours later they were subjected to being bitten by *S. ochraceum* adult fly on their backs. After the blood meal, the fed blackflies were collected and the microfilariae in them counted. It was observed that the microfilariae detected in the blackflies were 1/100 of those that were recovered from the vector that feed on the patients before they were treated with DEC. This study suggests a temporary immobilization of microfilariae by DEC.

Miura *et al.* [14] compared the *O. volvulus* microfilariae obtained from Mexico, Guatemala and Liberia, by scanning electron microscopy (SEM) and confirmed that they were all morphologically identical. They also revealed for the first time that the transverse annulations of the microfilariae ranged from 325 to 357. Tada *et al.* [15] counted the number of nuclei located between the cephalic space (CS) and the nerve ring of various species of microfilariae using the aceto-orcein squashing method. They concluded that this number of nuclei parameter obtained from the aceto-orcein squashing method could be generally applied to differentiating the various species of filarial nematodes microfilariae by extrapolating the counting method which was first proposed by Schacher *et al.* [16] for *Wuchereria bancrofti* microfilariae. Using this method, Mimori *et al.* [17] found no difference in the anterior nuclei index between Guatemalan and Nigerian microfilariae, with the former having 87.7 and the latter 88.0, respectively. Acid phosphatase patterns of *O. volvulus* microfilariae have long been used to classify the various strains involved. Ufomadu *et al.* [18] reported the presence of 13 different patterns of acid phosphates profiles among the *O. volvulus* microfilariae from Jos plateau, Nigeria. They proposed that the area contained a disproportionately high frequency of polymorphism among the Nigerian *O. volvulus* as compared with other areas, which usually showed only 4 or 5 different patterns of acid phosphates. Agatsuma and Ito [19] examined the patterns of 4 different isozymes of *O. volvulus* and *O. gutturosa*, with the aim of differentiating them. They found that LDH (Lactate dehydrogenase) isozyme pattern could be used for identifying the intra-species variation of the filariae. Furthermore, using the same technique, they also showed that it could be used to clarify the intra-species variation of *S. ochraceum* complex, which is the major vector for the transmission of onchocerciasis in Guatemala [20].

Epidemiological study

The first epidemiological study on onchocerciasis performed by Japanese parasitologists was that of Iwamoto *et al.* [21] in Illubabor Province, Ethiopia. They found that the prevalence of onchocerciasis among the 136 villagers of Abdella and 54 villagers of Didessa examined, were 51.4% and 66.7%, respectively. Besides dermatitis and presence of nodular lesions, a high prevalence of lower limb elephantiasis was noted among the villagers of these districts. To clarify the etiology of those lesions, Wonde *et al.* [22] examined histopathological sections of the inguinal lymph nodes of 10 elephantiasis patients. They observed abundant microfilariae and the consequent generalized fibrosis in the medullary cord of the lymph nodes of 9 patients. In contrast to the conclusions reached by other researchers on the etiology of the elephantiasis, they proposed that onchocerciasis was the major cause of the lesion. Moreover, Tanaka *et al.* [23] also reported that of a total of 975 *S. damnosum* collected at the Gojeb and Didessa riverbanks, between 10 to 40% of them harbored *O. volvulus* larvae, depending on the sites collected.

In Guatemala, Central America, Tada *et al.* [24] surveyed 4 plantations in onchocerciasis endemic area and found high prevalence of the disease among the inhabitants ranging between 46 to 68% by skin snipping. Nodule examination, which was traditionally used in this country, was considered low sensitive by this survey. The initiation of an ODA project by JICA in Guatemala in 1976, necessitated the compilation of a base line epidemiological data in San Vicente Pacaya (SVP), which was the focal point of the project. We showed that 1) 30.8% of the 2,153 inhabitants of SVP were positive for *O. volvulus* microfilariae, 2) the infection was prevalent in areas between 600–1,300 m above sea level, 3) the nodule positive rate and the microfilarial rates were proportional to each other in the individual plantation or village, 4) prevalence of onchocerciasis in man was higher than in woman with the former showing an MFD_{50} (Microfilarial Density) of 3.2 and the latter 0.9, and 5) the microfilarial rates in the skin were correspondingly proportional to that of the anterior chamber of the eye of patients [25, 26]. According to the epidemiological analysis of onchocerciasis infection in SVP, Yoshimura *et al.* [27] stated that the prevalence of the infection was 44.6% in man and 19.9% in woman in 1979, with the incidence being 0.08 in man and 0.04 of woman, respectively. Based on the field data obtained at SVP on onchocerciasis, Yanagawa *et al.* [28] proposed a modification of the epidemiological assessment for predicting the incidence of the diseases in the following 2 years after considering the frequency of false negatives in skin snipping. Wada [29] applied Muench's simple catalytic model in the analysis of

the epidemiological data of SVP. They concluded that 1) infection intensity correlated well with the density of the blackfly vector, 2) in low endemic area, the prevalence in man was higher than in women because the farmer went to work in highly endemic area where they were more prone to infection, and 3) in the highly endemic area, there was no difference in the prevalence between man and women.

Nwoke *et al.* [30] examined 1,821 inhabitants of Plateau Province in Nigeria, and found that the overall prevalence of onchocerciasis was 49.9%. The rate of infection was observed to increase with age. According to their observation of the clinical signs of the patients, pruritus was the most common, being found in 14.4%, depigmentation 9.9%, impaired vision in 6.4%, presence of subcutaneous nodule in 4.6%, and hanging groin in 0.8%. They also found cases of elephantiasis. Furthermore, Nwoke *et al.* [31] wrote a review on the characteristic features of the clinics of onchocerciasis in Jos province in Nigeria.

Clinical studies of onchocerciasis

Yamada [32] examined 759 inhabitants from various villages in SVP in Guatemala for the presence of ophthalmologic lesions: They observed that 6 to 27% of the villagers showed microfilariae in their anterior optic chamber; 10 to 66% showed corneal lesions and 25 to 67%, fluffy opacity. They reported similar occurrence of ophthalmologic lesions among the inhabitants of other endemic areas in that country [33–37]. Furthermore, when Nonaka *et al.* [38, 39] examined 1,259 inhabitants of SVP and the surrounding areas for dermatological related lesions, they found 149 persons with eczematous dermatitis, 290 with lower limbs depigmentation, 464 with lymphadenosis and 129 with pruritus. However, while they could not find any hanging groin or elephantiasis among the onchocerciasis patients, which were clinical signs reported in African onchocerciasis.

Aoki *et al.* [40] examined the incidence of onchocercal nodules among apparently nodule-free inhabitants of the high, medium and low endemic areas of onchocerciasis in Guatemala. During a period of 7 to 8 months, they found that incidence of onchocercal nodules among inhabitants of the high, medium and low endemic areas were 0.231, 0.083 and 0.022, respectively. Thus, they noted that the occurrence of nodule depended on the degree of endemicity in a certain area. Histological study showed that a nodule usually contained an average 0.6 adult male and 1.2 adult female, with 80% of the nodules greater than 10 mm in diameter releasing microfilariae into the cutaneous tissue.

Many papers on the efficacy and side effects of DEC in onchocerciasis had been published. Tada *et al.* [41] re-

ported on the histopathological changes seen in Mazzotti reaction, which has been frequently used for diagnostic purpose. According to them, a single dose of DEC could cause edematous change and dilatation of small vessels of the affected cutis. Furthermore, Mimori [42] concluded that the microfilaricidal action of DEC was based on the type I hypersensitivity and they also showed the formation of micro-abscess in the affected cutis. Sakamoto *et al.* [43] reported that the rise in peripheral blood leucocytes in the microfilaria-positive patients 3 days after administration of single dose of DEC, was mainly due to the increase in neutrophils.

As far as I know, there are several cases of Japanese parasitologists who were infected with onchocerciasis during their mission abroad but those infections were not reported. Yoshimura *et al.* [44] reported a case of Japanese who was infected with this parasite in Guinea and developed a nodule. The nodule was resected and confirmed histopathologically to be an onchocercal lesion.

Immunological and immuno-diagnostic studies

Ikeda *et al.* [45, 46] established an indirect hemagglutination test for the diagnosis of human onchocerciasis using crude extract of PBS of *O. volvulus* as well as the resolved solution of blood taken on filter paper. They observed a close relationship between the area endemicity and the presence of antibody in the inhabitants by this method. Using this test on those with onchocercal nodule and positive for microfilariae by skin snip in the endemic area, they showed that 98.3% of the subjects were positive for the antibody. The antibody positivity decreased with the reduction of infection scores. In non-endemic area, the antibody positivity among the inhabitants ranged between 2 to 3.4%. It was concluded that IHA positivity was proportional to the degree of endemicity of onchocerciasis and thus was applicable for epidemiological assessment. Furthermore, Korenaga *et al.* [47] applied the aforementioned antigen in an ELISA for the detection of *O. volvulus*-specific IgG and found that the result correlated well with the infection intensity. Tada *et al.* [48] did a cross-reactivity study using blood from patients infected with *Loa loa* and *Dipetalonema* sp. in an ELISA system and found high specificity for this onchocercal antigen. Hashiguchi *et al.* [49] examined patients with skin test by using a crude saline extract of *O. volvulus* and reported that 85.1% were positive for the test. The positivity of the test corresponded well to the length of the period after exposure to the infection, albeit the specificity of the test remained obscured. Furthermore, Takaoka *et al.* [50] examined 223 serum samples from microfilaria-positive patients by the double diffusion technique using crude

PBS-extracted *O. volvulus* antigen. They found that 93.1% of the sera tested were positive for the antibody. Moreover, they also observed a very high relative index of R = 0.977, between the presence for microfilaria in the skin and the presence of the filarial antibody as detected by the gel double diffusion method, in 5 villages in the endemic area. Kamiya *et al.* [51] developed a modified IHA (indirect hemagglutination antibody test) using adult worms obtained after digestion of the onchocercal nodule with collagenase as antigen. They then sensitized the formalin-fixed red blood cells with the adult worm antigen and found that the antigen-sensitized blood cells could be stored at −80°C for a long period of time. Ito *et al.* [52] demonstrated that crude antigen of *O. gutturosa* could be used in an ELISA to detect antibody to *O. volvulus* in humans. They observed a strong relation between the intensity of the infection with the ELISA results and also a high sensitivity of the test. Later Ito *et al.* [53] showed that the results of ELISA reflected the status of infection better in low endemic areas than the skin snip method. Moreover, Ito *et al.* [53] also reported that the ELISA result of inhabitants of the endemic areas do change markedly over a period of several months. For example, they found that 84 out of 101 patients showed the presence of antibody in the two tests carried out months apart but 5 out of 101 patients seroconverted from positive to negative or vice-versa during the two tests. Tada *et al.* [54] compared the various immune-diagnostic methods for onchocerciasis that were used in Guatemala and concluded that the IHA/ELISA showed reliable results, while the skin test showed many false positive cases. This was because the skin test showed many positive results among the group of inhabitants living in the endemic area that were still negative in skin microfilariae examination, whereas the IHA and ELISA pick up those with sufficient sensitization by parasite antigens. In general, it was noted that *O. volvulus* antigen was highly specific and did not show cross-reactivity with other filarial parasites.

Kawabata *et al.* [55] examined the relationship among circulation immune complexes (CIC), skin microfilarial density and the immune response by Raji cell radioimmunoassay. They observed that Guatemalan onchocerciasis patients with increased CIC concentration had lower microfilarial density and higher serum antibody titers against the worm. However, they also found that there was a suppression of the humoral immune response to tetanus toxoid as well as of the delayed hypersensitivity reaction to purified protein derivative of tuberculin (PPD) in the onchocerciasis patients. They concluded that CIC was involved in the modulation of immune responses in onchocerciasis. Akiyama *et al.* [56] reported that *O. volvulus* specific IgG

and IgE serum titers of microfilaria-positive patients were significantly higher than the serum titer of microfilaria-negative inhabitants or of that of healthy Japanese inhabitants in Japan. However, no difference in the IgA and IgM serum titers of both the microfilaria-positive patients and the microfilaria-negative patients were observed. When Korenaga *et al.* [57] examined the production of *O. volvulus* specific IgE among the inhabitants of onchocerciasis endemic area in Guatemala, they found that the IgE-ELISA value was lower in areas where the inhabitants showed higher microfilarial density. They also reported that in patients with higher microfilarial density, the lower their production of specific IgE was observed. Kawabata *et al.* [58] found increased level of IgM rheumatoid factor in 10 out of 57 Guatemalan onchocerciasis patients. They postulated that during the chronic stage of onchocerciasis, antigen stimulation might have led to autoantibodies production.

Nogami *et al.* [59, 60] produced 17 monoclonal antibodies to *O. volvulus* and examined their cross-binding activities to 13 species of filarial and non-filarial helminths (9 species of nematodes, 1 species of cestode, 3 species of trematodes) by indirect immunofluorescence. By cluster analysis, they observed that it was *Ascaris suum* and not other filarial parasites, that showed the most number of common antigens and thus the most closely related to *O. volvulus*. The schistosomes were antigenically least related to *O. volvulus*. Furthermore, by using *A. suum* antigen to screen against the monoclonal antibodies produced, Nogami *et al.* [60] were able to obtain 4 monoclonal antibodies that were specific for *O. volvulus* and characterized them.

Studies on the identity of *O. volvulus*

Brumpt [61] proposed a new species name, *O. caecutiens*, for the American isolate of the parasite but this proposal was rejected by most parasitologists due to lack of morphological and taxonomical evidence. However, the presence of autochthonous human onchocerciasis in Latin America had been supported by the finding of perforated holes in the cranium (which is frequently found among onchocerciasis patient), of the indigenous people who died before the arrival of Christopher Columbus at the New Continent [62, 63]. After their arrival at the New Continent, the Spanish recorded encountering many blind indigenous people there. Duke *et al.* [64] compared the various strains of *O. volvulus* from various endemic areas using cross compatibility test between the parasite and the blackfly vector combinations. This method was based on their concept of *Simulium/Onchocerca* complex. They applied this method to compare the uniqueness of the host/parasite

strains between that of West Africa and Central America, and vice-versa. They concluded that the two strains of *Onchocerca* from Africa and America, respectively, were markedly different from each other. With the support of the Overseas Research Grant from the Ministry of Education, Japan, that spanned 6 years since 1982, my colleagues and I attempted to clarify the identity of *O. volvulus*. We began by comparing the characteristics of the Central America strain (Guatemalan strain) of the parasite with that of the South American strain (Venezuelan strain). The latter strain had been considered as having its origin in Africa [65–67]. For this study, we compared the biometric and cytochemical features of the various strains of microfilariae, such as the number of nuclei in CS-NR (cephalic space-nerve ring) region and their acid phosphatase pattern. Furthermore, cross compatibility of *O. volvulus* microfilariae of each area to the *Simulium* vector of different area, karyotype of adult parasites and the clinical features of infected people of different areas were also compared. Based on previous reports, the chromosome of the African strain (n = 5, [68]) was considered different from that of the Mexican strain (n = 2, [69]). However, Hirai *et al.* [70] showed that the Guatemalan *O. volvulus* had a chromosome number of 2n = 6 + XX or XY and *O. gutturosa* had 2n = 8 + XX or XY. In a following study, when they compared the chromosomes of *O. volvulus* from Guatemala, Nigeria and Venezuela, they found that the chromosomes were all similar and thus concluded that the parasites belong to the same species [71]. Procunier and Hirai [72] wrote a review on the karyotype of *O. volvulus*. Takaoka *et al.* [73] studies the cross compatibility of *O. volvulus* microfilariae from Guatemala and Venezuela with *S. metallicum* of these two countries, by sending a Guatemalan patient to Venezuela to be infected with the vector and vice-versa. After studying the development of the larvae in the *S. metallicum* vector, they concluded that the two strains were identical or biologically very close. Biometric study of *O. volvulus* microfilariae from these countries also supported the aforementioned conclusion. Based on these evidences, we concluded that the Central American *O. volvulus* was imported from West Africa by the slave trade in the 16th–18th centuries [74, 75].

Studies on the transmission and control of onchocerciasis

Various studies on the biology of *S. ochraceum*, which is the major vector of *O. volvulus* in Guatemala, had been carried out. Matsuo *et al.* [76] found many *O. volvulus* infective larvae in the head of *S. ochroceum* that were kept at 25°C on day 8 post-microfilarial ingestion. However, only very few infective larvae were found in the blackfly that were kept at 20°C. Ito *et al.* [77] compared the *O. volvulus* transmission capability of the potential vector, *S. metallicun* with the major vector, *S. ochraceum*. They found that the vectorial capacity of the former was 1/3.5–1/5 that of the main vector. When Takaoka *et al.* [78] reared the *S. ochraceum* that had been allowed to feed on *O. volvulus* patient, at 10–30°C, they found that the temperature between 20–28°C was best for the growth of the ingested microfilariae and the subsequent larva stages. The time needed for the larval growth could be expressed by the equation $Y = -0.376 + 0.0222X$ (where Y is growth velocity and X is incubation temperature). When the temperature was alternated to simulate the night and day, growth of the larva was slightly retarded. The highest number of infective larva was found in the female blackflies that were reared at 22°C. However, the most efficient temperature combination for the larval growth was found to be 25°C during daytime and 16°C at night. Furthermore, Takaoka *et al.* [79] observed that even in *S. ochraceum* collected at a site 1,200 m or more above sea level, the growth of *O. volvulus* occurred slowly in the vector, reflecting the close relationship between ambient temperature and larval growth. In an epidemiological study at three Guatemalan plantations, Ochoa [80] confirmed the importance of *S. ochraceum* as the main vector of *O. volvulus*, with 0.33% of the captured female blackfly at Pena Blanca harboring infective larvae. Hashiguchi *et al.* [81] examined the degree of ingestion of *O. volvulus* microfilariae by the blackfly and the possibility of damage to the worm by the cibarial armature of the vector. Interestingly, they observed that the number of microfilariae taken in by the vector was much more than those estimated by skin snipping of the skin. They proposed that this might be due to the active movement of the microfilariae to the vector. With regard to this proposal, Tanaka *et al.* [82] pursue this point and found that the microfilariae intake by the vector blackfly was correspondingly dependent on the number of microfilariae from the skin of persons with moderate microfilarial density, but the intake of microfilariae from the skin of patient with low microfilarial density was disproportionately abundant. They assumed that some kind of vectorial stimuli might have attracted the microfilariae to the blood meal feeding site where they were taken up by the vector. A standard technique to assess vector susceptibility to *O. volvulus* that was established in Guatemala had been applied to other areas as well as to other species or strains of the vectors [83–86]. An interesting finding in Guatemala was the absence of infected persons in a village that was only 10 km away from the heavily *O. volvulus* infected zone that had an abundance of *S. ochraceum* [87].

Many studies on the ecology and biology of various

blackfly species in Guatemala had been published but due to space constraint, they were omitted in this section. Excellent reviews on this subject by Takaoka [88] had been published. A review paper written by Suzuki and Mizutani [89] on the studies of vector control in Guatemala is also available.

STUDIES ON ONCHOCERCIASIS IN ANIMALS

Taxonomical and morphological studies

Setaria spp. has long been suspected to be the causative agents of Wahi or Kose disease, which is characterized by localized alopecia and elephantiasis, in cattle in Japan. However, Niimi and Kouno [90] refuted this thinking and stated that the pathogen might be other filarial nematode. Later, it was Sato [91] who identified a filarial nematode obtained from the cervical ligament of cattle as *O. gutturosa*. He also identified the pathogenic filarial nematode, which causes "kasen" or, summer dermatitis in horses as *O. cervicalis*. Furthermore, Sato [92] also described the morphological features of the microfilariae of *O. equina*, *O. gutturosa*, and *O. cervicalis* in a comparative study. On the other hand, Kouno and Niimi [93] identified both the X and Y types of microfilariae found in cattle as that of *O. gutturosa*. They also compared the filaricidal activities of DEC and emetic tartar on the microfilariae and adult worms of *O. gutturosa* and concluded that both drugs were effective [94, 95]. Isshiki [96] reported that *O. gibsoni* was widely distributed in cattle in Korea. Of the 753 cattle examined in Gyeongsangnam-do and Gyeongsangbuk-do, Korea, 9.8% were found infected with *O. gibsoni*. He later published the morphometrics of the adult worms and microfilariae of that species [97–99].

Clinical and epidemiological studies

Niimi and Kouno [100] attributed the pathogenicity of Kose disease seen in cattle in Japan to the allergic reaction caused by the microfilariae in onchocercal infection. In Guatemala, besides conducting their research on human onchocerciasis, Hashiguchi *et al.* [101] also examined the prevalence of onchocercal infection in cattle and horses in that country. They found that 50–79% of the horses and 23–93% of the cattle examined harbored microfilariae in their cervical ligament and skin, respectively. The respective microfilariae were identified as *O. cervicalis* in the horse and *O. gutturosa* in the cattle.

Human infection with zoonotic *Onchocerca* spp.

Hashimoto *et al.* [102] reportedly found a filarial worm in a nodule of the left foot of a 2-year old girl in Oita, Japan. To clarify the route of transmission of *Onchocerca* to that little girl, Takaoka *et al.* [103] examined several species of blackfly such as *S. bidentatum* in the cattle farm at the vicinity of the girl's home. They found natural infection of *O. gutturosa* and *O. lienalis* larvae in *S. bidentatum*. Later, Takaoka and Bain [104] examined the X and Y types of larvae found in blackflies that were allegedly produced by *O. gutturosa*. They concluded that the Y type probably belongs to *O. lienalis* and the X type to a still unknown species. Furthermore, they also conducted a survey on the prevalence of onchocercal infection in animals in the Tohoku area, Japan. Since they found 4 different species of blackfly harboring *Onchocerca* larvae, they suggested the possibility of human infection by zoonotic *Onchocerca* spp. through transmission by blackflies.

To shed light on the possibility of human infection with animal *Onchocerca* in Kyushu, Takaoka [105] examined 19,005 blackflies, collected from several cow barns in Oita and Kumamoto Prefectures, for natural infection with *Onchocerca*. He found that *S. bidentatum* was the dominant species that harbored type I and II larvae (which were considered to be *O. gutturosa*) while the *S. kyushuense* and *S. arakawae* harbored the type III larvae that were thought to be *O. lienalis*. Moreover, to examine the possibility that *Culicoides* might be involved in the transmission of *Onchocerca*, Takaoka and colleagues dissected 17,006 midges that were collected using light trap. No onchocercal infection was seen in the 8 species of *Culicoides* examined [106]. Recently, Takaoka *et al.* [107] reported a 57-year old woman in Oita prefecture being infected with an immature female worm of *O. gutturosa* in her wrist. These evidences suggested the possible infection in man with zoonotic *Onchocerca*, despite the episode being an incidental one.

Conclusion

With the introduction of a potent anti-filarial drug, namely, ivermectin (also known by the brand name, mectizan), the strategy for the control of onchocerciasis worldwide had shifted to mass therapy. This drug is also being used in Central and South Americas on a national basis to control the disease. It is not off the mark to say that the foundation for establishment of the basic techniques and development of human resources for the control of onchocerciasis in Guatemala had been laid by the onchocerciasis research project sponsored by JICA. Thus, by conducting research in that country, Japanese researchers and Japan's ODA had contributed in away, to the fight against onchocerciasis for the realization of a healthy population in Guatemala.

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