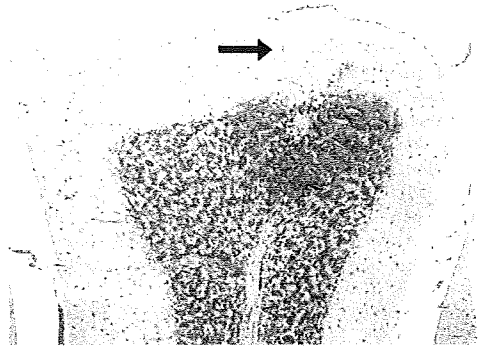

Fig. 3. A fresh haemorrhagic lesion in the granular layer of the cerebellum in gerbils 24 h after infection with *Baylisascaris procyonis*; larva (arrowed) in the molecular layer of cerebellum immediately beneath the pia mater.

7 days in the case of *A. suum*. On day 7, haemorrhagic lesions in the lungs were the most prominent feature in gerbils infected with both species, although these lesions gradually disappeared and no larvae were recovered thereafter from any organ.

As far as the migration route is concerned, the highest recovery rate in the gastrointestinal wall occurred in *T. canis*-infected gerbils followed by *B. procyonis*-, *A. suum*-, and *B. transfuga*-infected gerbils 6 h after infection. Larvae of both species of *Ascaris* immediately migrated away from the intestinal wall to the liver within 24 h after infection, with *Toxocara* and *Baylisascaris* larvae remaining there until the end of experiment. *Ascaris lumbricoides* larvae were minimally recovered from not only the gastrointestinal tract but also from other organs throughout the experiment. The recovery rate of ascarid larvae from the brain was high in the case of *T. canis* (3.1%) and *B. procyonis* (2.6%) at day 7 post-infection as compared with *B. transfuga* (0.7%), even though *B. procyonis*-infected gerbils did not survive until the end of the experiment. On the other hand, no *A. lumbricoides* larvae were found and only one *A. suum* larva was observed on day 7 post-infection. The recovery rate from skeletal muscles was high in the case of *T. canis* and *B. transfuga*, although the number of muscle stage larvae of *B. transfuga* was always higher than that in *B. procyonis*.

Discussion

Takayanagi *et al.* (1999) demonstrated that the Mongolian gerbil is a suitable animal model for ocular toxocariasis because of the high incidence of ocular invasion by the larvae. However, little is known about the migratory behaviour or pathogenesis of ascarid larvae in gerbils. In the present study, *T. canis* larvae migrated to the liver within 3 days after infection, and were thereafter distributed equally in skeletal muscles and the brain. These results are similar to those of Olson (1962) and Sprent (1952), suggesting that the migration route and final site of infection have little influence on the development of ocular toxocariasis in gerbils.

In the present study, *B. procyonis* larvae more so than *B. transfuga* were likely to accumulate in the brain and all gerbils infected with *B. procyonis* died from severe neurological disturbances within 2 weeks after infection. On the other hand, gerbils infected with *B. trasfuga* survived throughout the duration of the experiment, despite exhibiting neurological disorders. The number of *B. transfuga* muscle stage larvae was always higher than in *B. procyonis*-infected gerbils. Sato *et al.* (2004) reported that the *B. procyonis* and *B. transfuga* larvae that had migrated into the brain of gerbils were larger than those of *T. canis*; however, no significant differences in larval size were observed between *B. procyonis* and *B. transfuga*. These results suggest that severe neurological disorders caused by *B. procyonis* could be attributed to the total amount of larvae in the brain. Additionally, these findings suggest that *B. procyonis* larvae may have a neurotropism, whereas *B. transfuga* larvae may have an affinity for muscular tissue. Further studies are needed to better understand the pathogenetic differences between *B. procyonis* and *B. transfuga* larvae in the brain of infected gerbils. Ophthalmologically, the lesions elicited by both species closely resembled each other although the incidence was extremely low in *B. transfuga*-infected gerbils. These results indicate that *B. transfuga* should not be used as an alternative parasite for studying diffuse unilateral subacute neuroretinitis induced by *B. procyonis* in gerbils (Akao *et al.*, 2003).

In the present study, the infectivity of *A. suum* and *A. lumbricoides* in gerbils was very low, with migration to the central nervous system being minimal and no ophthalmological changes were found. Therefore, *A. suum* and *A. lumbricoides* are considered inappropriate parasites for studying ophthalmological and neurological disorders in gerbils. Severe to mild pulmonary haemorrhagic lesions were common in infected gerbils, although a complete healing of these lesions occurred in the case of *A. suum* and *A. lumbricoides*. Interestingly, no larvae were recovered from any organs of these gerbils beyond 14 days post-infection. Mouse models have shown a similar pattern (Slotved *et al.*, 1997, 1998). To further document the migratory behaviour of *A. suum* and *A. lumbricoides* larvae in gerbils after 7 days of infection, the contents of the gastrointestinal tract were examined daily between days 8 and 13 post-infection because we assumed that the larvae might return to the intestine via the larynx and pharynx. However, no larvae were detected (data not shown), suggesting their rapid expulsion from infected gerbils.

Further studies are needed to more fully elucidate the migration behaviour and pathogenesis of *T. cati* so that we may potentially improve the therapy against this important zoonotic parasite of human VLM (Akao *et al.*, 2000; Fisher, 2003).

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Identification of Human Herpesvirus 6 in a Patient With Severe Unilateral Panuveitis

Human herpesvirus 6 (HHV-6) is a member of the HHV family[1] and has been associated with immunodeficiency disorders and neurologic diseases.[2] This widespread virus can be classified into 2 groups: variant A (HHV-6A) and variant B (HHV-6B).[2] Although HHV-6B is the known causative agent in exanthema subitum,[3] the association of HHV-6A with clinical entities is still unknown. We describe a patient with severe right-sided panuveitis and multiple subretinal lesions. The *HHV-6A* genome was detected in the ocular fluid of this patient.

Report of a Case. A 75-year-old man developed a sudden decrease in vision in the right eye in 2005. Slit-lamp examination of the right eye disclosed ciliary hyperemia, moderate mutton-fat keratic precipitates, and severely inflamed anterior chamber cells with hypopyon. Funduscopic examination of the right eye revealed dense vitreous opacities, optic disc swelling, yellowish-white massive retinal lesions measuring approximately 1.5 optic disc diameters, and whitish retinal exudates (**Figure 1**). The left eye was normal. Results of all systemic examinations, including serologic testing for human immunodeficiency virus, were negative, and results of serologic testing for HHVs (herpes simplex virus, varicella zoster virus, Epstein-Barr virus, cytomegalovirus, and HHV-6) were positive except for varicella zoster virus. On the basis of the ocular manifestations, a viral infection was suspected. After informed consent was obtained, an aliquot of aqueous humor and an aliquot of peripheral blood were collected and examined for further investigations. Immunoglobulin G for *Toxocara* larval excretory-secretory antigen in the aqueous humor and serum was detected using an anti-*Toxocara* antibody detection kit.[4] A multiplex polymerase chain reaction demonstrated HHV-6 genomic DNA in both samples but not other HHVs (herpes simplex virus type 1 or 2, varicella zoster virus, Epstein-Barr virus, cytomegalovirus, HHV-7, or HHV-8). To acquire quantitative data, a real-time polymerase chain reaction was performed at different stages of the clinical course. In the acute phase with active inflammation, a high copy number for the HHV-6 DNA was detected in the samples (aqueous humor: 2.4×10^6 copies/mL; serum: 5.4×10^6 copies/mL). Because the patient indicated that there was progression of intraocular inflammation, right eye diagnostic pars plana vitrectomy was performed. A high copy number for the *HHV-6* genome was detected in the vitreous fluid, retinal membrane, and peripheral blood mononuclear cells. In addition, IgG for *Toxocara* larval excretory-secretory antigen in the vitreous was also detected. These data led us to make the diagnosis of panuveitis related to a *Toxocara canis* larva or an HHV-6 infection. Next we examined whether the HHV-6 infection was indicative of variant A or variant B. A high number of copies of HHV-6A was detected in the samples, and the *HHV-6A* genome decreased after antiviral valganciclovir hydrochloride treatment associated with systemic corticosteroids, whereas the *HHV-6B* genome was not detected (**Figure 2**). After treatment, funduscopic examination of the right eye revealed resolution of the vitreous opacities, optic disc swelling, and retinal exudates.

Comment. It is difficult to be certain whether HHV-6 was the causative agent in intraocular inflammation in this patient. Anti-*Toxocara* antibodies were also detected in serum and aqueous humor and vitreous samples, the significance of which is difficult to interpret. Another hypothesis could be that HHV-6 favored *Toxocara*-generated inflammation. However, the vi-

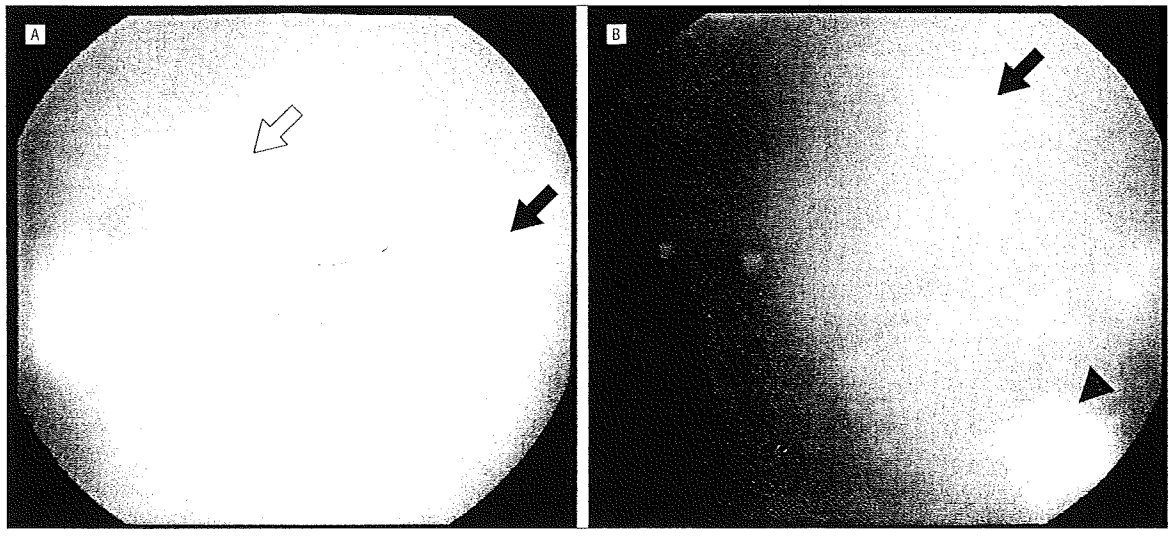

Figure 1. Fundus photographs of the right eye of a patient with a human herpesvirus 6 variant A infection. A, Whitish retinal exudates (white arrow), optic disc swelling (black arrow), and dense vitreous opacities are seen. B, Retinal yellowish-white massive lesions (black arrowhead) and optic disc swelling (black arrow) are seen.

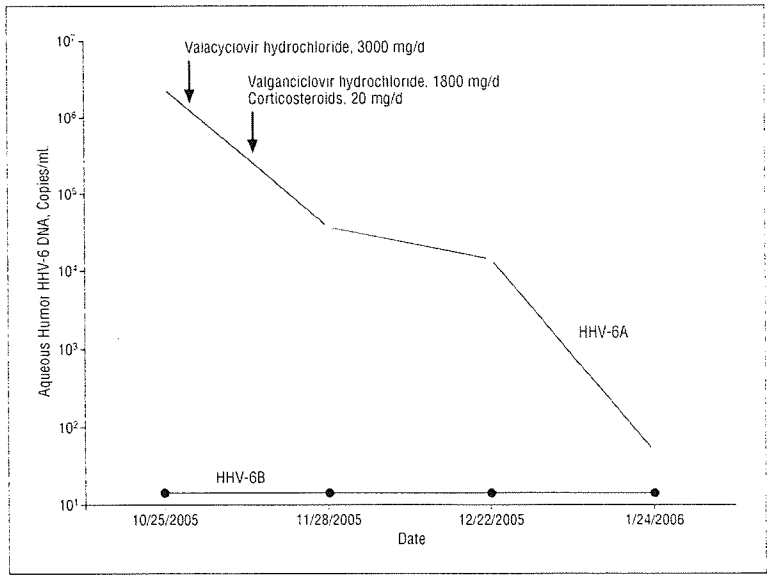

Figure 2. Serial measurement of aqueous humor human herpesvirus 6 variant A (HHV-6A) and variant B (HHV-6B) DNA levels by means of real-time polymerase chain reaction..

ral DNA and intraocular inflammation decreased in response to antiviral agents, suggesting that HHV-6A has some role in the pathogenesis of the ocular inflammation. To our knowledge, this is the first report of a case of HHV-6A associated with intraocular inflammation. These observations suggest that HHV-6A infection may have a role as a causative agent in severe intraocular inflammation.

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Severe Darkening of a Facial Skin Graft From Latanoprost

Latanoprost is a 17 phenyl–substituted analogue of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which decreases intraocular pressure by increasing uveoscleral outflow. Since its introduction as a topical eye medication, several authors have reported adverse effects, like subtle hyperpigmentation of periocular skin and eyelid-margin hyperemia.[1] Herein, we present a case of a patient using latanoprost who developed severe darkening in a facial skin graft.

Report of a Case. A 68-year-old woman was diagnosed with primary open-angle glaucoma in September 2002. Topical latanoprost was commenced in both eyes, with a good control of intraocular pressure. In April 2005, a malignant melanoma was surgically excised from the left side of the patient's face and skin was grafted to this area from her neck behind the ear. Histology confirmed a low-risk, superficial, spreading malignant melanoma in situ, which was excised with adequate margins. In September 2005, severe darkening of the skin graft was noted together with subtle bilateral periocular hyperpigmentation and eyelid-margin hyperemia (**Figure 1**). Her medication was switched from latanoprost to topical brinzolamide in both eyes with a good control of the intraocular pressure. One month after stopping latanoprost, the skin graft had lightened significantly and the subtle bilateral periocular hyperpigmentation and eyelid-margin hyperemia had resolved (**Figure 2**).

Comment. Prostaglandins increase both melanocyte dendricity and melanin synthesis in the skin. Prostaglandin $F_{2\alpha}$ stimulates the activity and expression of tyrosinase, the rate-limiting enzyme in melanin synthesis, and the $PGF_{2\alpha}$ receptor has been shown to be up-regulated by UV radiation in melanocytes in vitro and in human skin in vivo.[2] Researchers have shown how proteinase-activated receptor 2 in keratinocytes plays an important role in skin pigmentation. Activation stimulates uptake of melanosomes through phagocytosis and also stimulates release of prostaglandin E_1 and $PGF_{2\alpha}$, which stimulate melanocyte dendricity.[3] Prostaglandins have also been implicated in postinflammatory skin hyperpigmentation.[4]

Significant lightening of the skin graft together with the resolution of subtle bilateral periocular hyperpigmentation and eyelid-margin hyperemia 1 month after stopping latanoprost implies that a local adverse drug reaction to latanoprost occurred in this patient. Absorption of latanoprost into facial skin is likely to occur from tear spillover during topical application. The severe dark-

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Review

Toxocariasis in Japan

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Abstract

Toxocariasis has long been considered a parasitic disease affecting pet owners and children who often play in sandboxes at public parks. Recent cases of this animal-borne infection, however, indicate that its clinical manifestations and etiologies are changing. In this article, we will describe the critical characteristic features of toxocariasis alongside the contributions of Japanese researchers to a better understanding of the disease.

Keywords: *Toxocara canis*; *Toxocara cati*; Toxocariasis; Visceral larva migrans

Contents

1. Introduction

Among animal-borne diseases, toxocariasis is one of the most popular parasitic infections in the world, caused by the larval stage of *Toxocara* spp. Humans are infected mainly by the tiny developmental stage of the parasite, which belong to the family Ascaridoidea, through their pet dogs and cats. Other natural hosts include wild Canidae for *Toxocara canis* and wild felines for *Toxocara cati*. Symptoms depend on organs affected and the magnitude of infection. It is usually a non-fatal disease, but the larvae migrate through the eyes and can cause severe vision disability or even blindness.

In 1950, Dr. Wilder, an American ophthalmologist, histopathologically identified a nematode of unknown etiology in the retinas of 26 out of 46 enucleated eyes with retinoblastoma [1].

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Two years later, Beaver et al. [2] recognized the same parasite in the liver of three young children. Shortly afterwards, the parasite was correctly identified as an infectious stage larva of *T. canis* [3–5]. Since then, many clinicians and biologists have been accumulating knowledge of *Toxocara* and toxocariasis.

In this review article, we describe the lesser-known contributions of Japanese researchers to the understanding of *Toxocara* and toxocariasis. This articles builds on the work of Kondo [6], focusing on the topics that he did not cover in his review and on new findings since his publication.

2. Toxocariasis in humans

2.1. Clinical cases

Toxocariasis is clinically classified into four types: visceral, ocular, neurologic, and covert [7,8]. In 1963, the first report on toxocariasis in Japan was presented orally at the 32nd Annual Meeting of the Japanese Society of Parasitology by Fushimi et al. [9]. A 14 year-old boy was admitted to a university hospital because of fever, hepatomegaly and persistent eosinophilia. The patient died from severe anemia six months later. Though no autopsy or serological examinations were performed, the patient was strongly suspected to have suffered from visceral toxocariasis. In the early 1960s, immunological tests for parasitic infections, especially for helminthiasis, had only just begun, and antigen for the diagnosis of toxocariasis was not yet known.

Just as in other parasitic infections, direct demonstration is the only way to make definite diagnosis of toxocariasis. However, it is difficult to find the larva in either tissue biopsies or autopsies due to its very small size. So far in Japan, one morphologically and two pathologically confirmed cases have been reported [10–12]. Two additional reports, both of ocular toxocariasis, were doubtful because of the lack of characteristic features of the parasite; the authors nevertheless reproduced the microscopic findings of the purported larva in their papers [13,14]. One of these two cases showed increased antibody production in vitreous fluid against *Toxocara* antigen prepared from larval excretory–secretory product (LES), suggesting that the case might be attributable to ocular toxocariasis.

Serology is an alternative method for the diagnosis of toxocariasis. A method has been established for *in vitro* cultivation of the larvae, with LES prepared from the culture medium serving as an antigen. Detection of specific antibodies against LES provides evidence of *Toxocara* infection in individual patients and useful tool for understanding the epidemiological characteristics of this disease. The first serological survey in Japan was reported by Matsumura and Endo [15] using sera of 83 clinically healthy children. In their sample, 3.6% tested were positive for LES. In another study, Matsumura and Endo [16] demonstrated that 20 of 530 adults possessed the IgG antibody to LES. The positive individuals were thought to have a latent or past infection. In a large-scale seroepidemiolgical survey, Kondo et al. [17] collected 3277 sera from 14 prefectures in Japan and tested for LES antibodies. Antibodies were confirmed in 52 individuals (1.6%), but geographical patterns were notable: the highest prevalence rate was observed in Miyagi Prefecture (6.1%), and the lowest was in Ibaragi Prefecture (0.5%). The researchers concluded that the overall seroprevalence rate was in good agreement with those reported from other countries [17–19].

Based on improvements in the field of serology, diagnosis of toxocariasis is usually made by detection of the specific antibody to LES, along with clinical manifestations such as eosinophilia, eosinophilic pneumonia, or ophthalmoscopic findings.

2.2. Characteristic features of toxocariasis

2.2.1. Toxocariasis as a food-borne infectious disease

Using serological methods, there were nearly 200 reports of toxocariasis in the database of Japana Centra Revuo Medicina, and almost 300 cases have been diagnosed in Japan in the past two decades. Among these cases, some significant reports have provided a new perspective on the pathogenic mechanisms of toxocariasis.

Since Beaver et al. [2] introduced the concept of visceral larva migrans, characterized by chronic eosinophilia with granulomatous lesions in the liver, toxocariasis was regarded as a disease in children who were infected by soil contaminated with embryonated eggs [20]. In 1983, Sakai et al. [21] reported a case toxocariasis after ingestion of raw chicken liver. The 57-year-old man was admitted to a hospital due to cough, fever and weight loss. Complete blood count revealed a marked increase in eosinophils in peripheral blood with leukocytosis, and serum antibody against *T. canis* was strongly positive. Before onset, he and his friends had eaten raw chicken livers derived from his poultry and boar farm. Soon after the meal, they experienced abdominal pain, vomiting and diarrhea, but the symptoms improved within two days after ingestion. One month later, his chief complaints emerged. Two similar cases were subsequently reported by the same group [22].

These cases clearly indicates that the disease should be considered a food-borne parasitic infection. Four additional papers describing six patients were published in Japan in the 1980s [22–25]. These patients, all male and between 22 and 51 years of age, had a history of eating raw meat or liver of fowl and/or cattle before onset of symptoms. The possibility that raw liver of domestic animals can transmit the pathogens of human visceral larva migrans was substantiated by Lee et al. [26] of Yonsei University College of Medicine in Korea. They found that a dietary habit of raw liver was much more frequently seen in males than in females, especially in the 31–40 age group. Experimental studies revealed that chicken, cattle and swine were able to act as paratenic hosts for *T. canis* [27–29]. Most of the adult cases reported in recent years in Japan are categorized as this type of infection [30].

2.2.2. Respiratory illness and toxocariasis

In animal models in rodents, hatched larvae migrate into the lungs through the liver after ingestion, resulting in liver dysfunction and pneumonia [31–33]. In humans, similar manifestations are well documented in the literature [30,34–36]. Pulmonary lesions appear on computed tomography as multifocal subpleural nodules with halos or ground-glass

opacities and ill-defined margins. Additionally, transient pulmonary infiltrates are a characteristic finding. Morimatsu et al. [30] recently reported a familial case of visceral toxocariasis after consumption of raw chicken livers. In this case, the patients, a father (71 years old) and his son (45 years old), ate raw chicken livers three weeks before onset and then developed mild fever, general fatigue, headache and respiratory disorder. The specific antibody to LES was identified both in their serum samples and in bronchoalveolar lavage fluid (BALF). *T. canis* larvae were recovered from chicken liver from the same source as that ingested by the patients. These cases showed that BALF is a reliable specimen to demonstrate LES antibodies when the patient shows respiratory illness.

2.2.3. *Urticaria-like skin lesions and toxocariasis*

Parasitic infection is often said to be associated with chronic urticaria [37]. This is still a controversial issue, but acute urticaria is certainly associated with infection with larva from the marine fish parasite, *Anisakis simplex* [38]. Japanese have long tradition of eating raw fish, sashimi and sushi, and anisakidosis is a common parasitic infection in Japan. It is well documented that urticaria is closely related to the infestation of *Anisakis* larva [38,39]. As with anisakidosis, an allergic reaction could be elicited by the invasion of *Toxocara* larvae and result in skin rash that looks like hives. These skin manifestations might occur as a result of immunological response to larval metabolites [40,41].

In 1999, the first confirmed case of toxocariasis with larva in subcutaneous tissue was reported [11]. A 26-year-old female with fever, headache, and dry cough was admitted to a university hospital. Her peripheral blood smear showed an eosinophilia (61%) and her chest radiograph revealed multiple nodules. A diagnosis of visceral toxocariasis was made after detection of LES antibodies. During her hospitalization, several brown itchy nodules, which were thought to be prurigo, developed on her legs. Histological examination showed *Toxocara* larva in the center of an eosinophilic and lymphocytic abscess. The patient admitted frequently eating raw beef liver almost one year before her hospitalization for its purported health benefits. We can learn from this case that larvae migrating into subcutaneous tissue directly elicit pruriginous skin lesions.

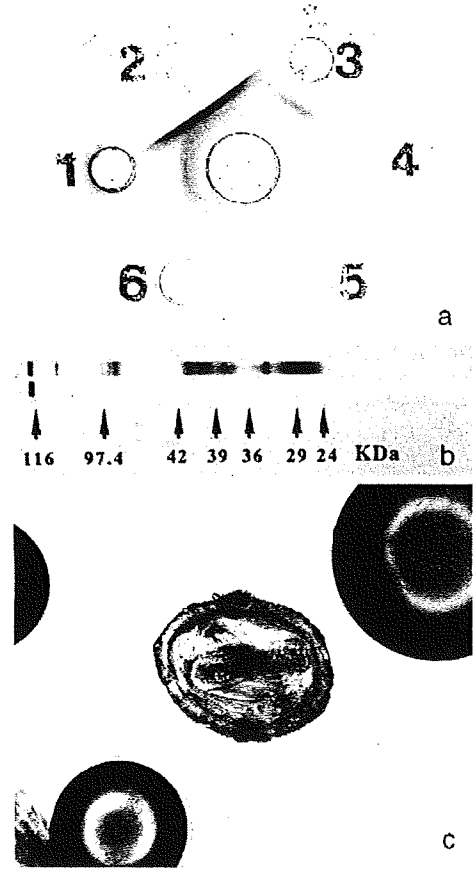

Fig. 1. The results of double gel diffusion (a) and western blot (b) tests of a patient of visceral toxocariasis. Strong precipitin bands were obviously observed between larval excretory–secretory products (LES) of *Toxocara canis* and patient's serum by means of double gel diffusion test. Antigens used in this test were adult worm extract (AEX) of *T. canis* (1), LES of *T. canis* (2), AEX of *Dirofilaria immitis* (3), AEX of *Ascaris suum* (4), LES of *Anisakis simplex* (5) and AEX of *Ascaris lumbricoides* (6). Western blot test shows a whole range of LES molecules were reacted with the patient's serum (upper strip) but not with a normal control serum (bottom strip). An embryonated egg recovered from the soil in the park where the patient often played (c). A fully developed and live *Toxocara* larva was found in the egg.

2.2.4. *Toxocariasis is a disease that affects adults rather than children*

Many reviews from western countries indicated that children under 12 years old, who often play outside, are the most affected age group for toxocariasis [42,43]. They are accidentally infected with *T. canis*/*T. cati* eggs, which expelled in feces puppies and fully develop in the surrounding environment within two to four weeks. Therefore, contaminated soil is the most important etiological source for toxocariasis [44,45]. Hori et al. [46] reported a case of visceral toxocariasis in a 1.5-year-old girl with fever, hepatomegaly, and eosinophilia (73%). The patient had a history of pica, particularly eating soil from a nearby park where she frequently played with her brother. Serological examination strongly suggested that she was suffering from *Toxocara* infection (Fig. 1a, b). They also found many embryonated eggs from the soil in the park that contained a live larva closely resembling *T. canis* eggs (Fig. 1c). Fortunately, her brother showed a negative result in serological tests.

In a review article of Barriga [47], the average age of visceral toxocariasis was 9.5 years, and only 18% of patients were adults. However, in recent investigations, adults rather than young children were more frequently affected by this parasite. This tendency is particularly true for ocular toxocariasis. Yoshida et al. [48] described that, among 38 Japanese cases of ocular toxocariasis, 34 (89%) were older than 20 years of age, and suggested that clinical features observed in these patients were somewhat different from those of previously reported cases [49]. Therefore, ocular toxocariasis is no longer merely a disease of young children, but affects any age group having a risk factor such as consumption of raw meat or close contact with contaminated soil.

As of the end of 2006, 584 clinically suspected cases of toxocariasis (112 of visceral type and 472 of ocular type) have been referred to our laboratory for detection of the anti-*Toxocara* antibody. We omitted 109 cases from this study due to a lack of description of the patient's age and sex. In visceral toxocariasis, the male-to-female ratio in the remaining sample was 2.04 (male: 53, female 26). The average age was 39.2± 21.7 (range, 0–83 years old) in male and 31.3±23.9 8range, 0.5–82 years old) in female. On the other hand, the male-to-female ratio in ocular toxocariasis group was 1.16 (male: 213, female: 183). The average age was 39.3±18.5 among males (range, 2–83 years old) and 37.6±18.2 among females (range, 2–74 years old). There were no significant differences in age distribution between males and females (Fig. 2). A similar result was obtained by Fujino et al. in 1998 [50].

2.2.5. Myelitis and toxocariasis

According to the case-control study by Magnaval et al. [51], migration of *T. canis* larvae in the human brain does not frequently induce recognizable neurological signs, but is possibly responsible for repeated low-dose infections. These light parasitic burdens usually do not appear to elicit a special clinical symptom, but in some cases, sever neurological disorders such as encephalitis, myelitis and meningitis are manifested [52]. In Japan, Ota et al. [53] reported a case of eosinophilic meningo-encephalo-myelitis due to *Toxocara* infection. The patient, a 21-year-old woman, showed frontal headache, low-grade fever and convulsion. She had a long history of close contact with her pet dog. Immunological tests were strongly positive for LES antigen in both her serum and cerebrospinal fluid. Based on clinical evidence and characteristic features in similar patients, Kira and his colleagues proposed a new disease entity: "atopic myelitis" or "parasitic myelitis." They assumed that allergic reaction to LES might be involved in this neurologic disorder [54]. Interestingly, most of the patients lived in Kyushu District, in the south of Japan, suggesting that myelitis due to *Toxocara* infection might be a regional clustering disease.

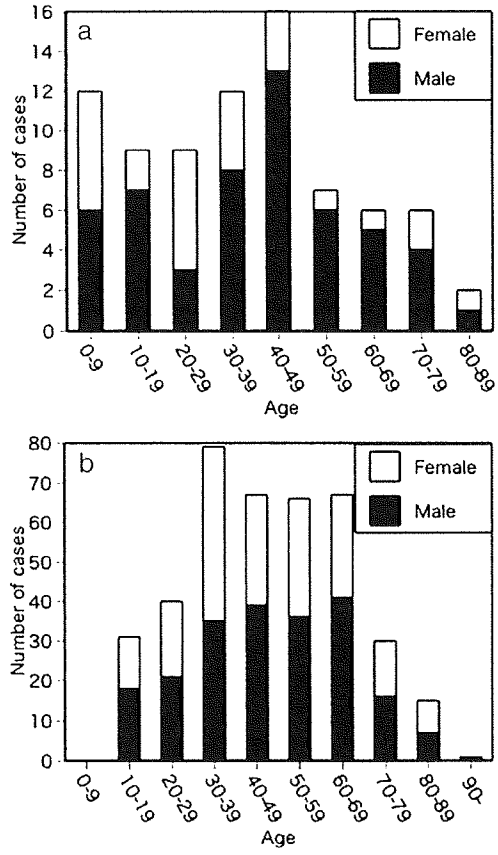

Fig. 2. Age distribution of suspected cases of visceral (n=79)(a) and ocular toxocariasis (n=396)(b) referred to our laboratory from August 1994 to December 2006.

2.3. T. cati

Because morphological differences between *T. canis* and *T. cati* in the adult stage are apparent [55], *T. cati* is easy to identify when patients expel adult worms. It has been suggested that *T. cati* could develop in children through the ingestion of the immature worm of *T. cati* [56]. More than 26 cases were reported so far [56,57], but there was only one case was reported from Japan. A 5-year-old male boy was admitted to a hospital due to a complaint of vomiting 3 worm-like foreign bodies. These worms were morphologically identified as two female and one male immature worms [58].

On the contrary, there are few reports of human intestinal infection with adult worms of *T. canis* [59], and many of these are believed to be erroneous observations [60]. Serological discrimination between toxocariasis canis and toxocariasis cati, however, is not so apparent, because of complete cross-reactivity between the two LESs, although *T. cati*-specific LES has been identified [61]. Therefore, distinguishing between *T. canis* and *T. cati* is even more difficult if somatic antigens are used in the serological diagnosis [62–64]. For the precise serodiagnosis of toxocariasis, a great deal of additional research effort is needed to obtain *T. cati*-specific LES antigens.

3. Advances in serological diagnosis

3.1. Antigens

As mentioned above, the most reliable and suitable antigen for the diagnosis of toxocariasis is LES from *T. canis*. Once the larvae are cultivated *in vitro*, they are viable for up to two years. During this period, no morphological changes have been observed, but chemosusceptibility to some compounds were found to have changed [65], suggesting that the physiological natures of the larva do change over this time period. The nature of LES was extensively studied by Maizels and colleagues [61,66–68]. Around the same time, Sugane and Oshima demonstrated that LES had an ability to induce not only IgG and IgM antibodies, but also IgE antibody in mice. Allergenic activity was lost when LES was treated with guanidine hydrochloride and 2-mercaptoethanol. LES also showed a cross-reaction with serum from *Ascaris suum*-infected mice

[69]. In addition, studies have identified numerous lectin-specific glycoconjugates on the surface of the larvae [61,66–68,70–73], and these have been found to dynamically change during the course of infection in murine [74] and rabbit models [75].

Although the antigenicity and specificity of LES is fairly high, cross-reaction to other parasites, especially nematode parasites, have been observed [76]. To overcome this problem, Yamasaki et al. [77] produced a recombinant antigen that reacted with serum from patients with toxocariasis but not from those with roundworm or hookworm infections.

3.2. Rapid diagnostic test for toxocariasis

For many years, numerous diagnostic measures, such as the double gel diffusion test, immunoelectrophoresis, indirect hemagglutination test, latex agglutination test, plate-based ELISA, membrane-based dot-ELISA, etc., have been employed to detect specific antibodies against LES. However, these tests require 1.5 hours or more to obtain an accurate result. In 1997, a new rapid diagnostic test kit for the detection of anti-LES antibody was introduced by us [78]. The test is based on the antigen-sensitized nitrocellulose membrane-based assay. It is easy to perform, does not require any sophisticated apparatus or expertise and the results can be obtained within 3 min. This test kit can even detect the antibody in intraocular fluid.

4. Conclusion

In this review article, we present an overview of human toxocariasis in Japan. Due to space limitations, we do not describe in detail the aspects of experimental investigations concerning biology, immunology and molecular biology using animal models. However, we briefly pay special attention to Japanese investigators who contributed to advance the understanding of toxocariasis. In early studies, Oshima established a standard method for the oral inoculation of eggs, in which the albuminoid coat of the egg is first removed in order to prevent the adhesion of eggs onto glassware [79]. Sugane is a longtime co-worker of Oshima, and his colleagues are actively engaged in the field of immunology [80–88]. They demonstrated many examples of cellular immunity to *Toxocara* infection in mice. The late Dr. Tsuji made pioneering efforts to develop immunodiagnostic techniques for toxocariasis [50,89,90]. Recently, Mongolian gerbils, *Meriones unguiculatus* have been established as a suitable animal model for experimental ocular and neurologic toxocariasis [91–94].

Human toxocariasis is a public health hazard not only in children but also in adults, both in developing and developed countries. There are still questions to which we have no answers: How does ocular toxocariasis develop? Why do nearly half of ocular toxocariasis patients not produce detectable antibody to LES? What is the pathogenesis of neurologic toxocariasis? What mechanisms are involved in the reemergence of *Toxocara* larvae during pregnancy both in definitive and undefinitive hosts? In addition, we have not yet established an effective anthelmintic against *Toxocara* parasites in the tissue stage, especially for the ocular toxocariasis. Continuous efforts should be made to address these issues. Finally, toxocariasis is a disease that afflicts two of the very best and oldest friends of humans: dogs and cats. Therefore, we must continue to study this puzzling disease both for the sake of humans, and for that of our animal friends.

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REVIEW ARTICLE

Naohide Takayama

Rabies: a preventable but incurable disease

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Abstract Rabies is a typical zoonotic disease which has been known for more than 4300 years. To date, no effective medical therapy has been established for overt rabies. The rabies post-exposure prophylaxis (PEP), which is a serial vaccination against rabies starting as soon as possible after the patient was bitten by a suspected rabid animal, is the only way to prevent death. In Japan, no rabies case has been reported for about 50 years. However, rabies is epizootic in many Asian countries, where more than 50% of the rabies deaths in the world occur. The Japanese travelers who visit these countries every year may not be aware of this fact since no rabies occurs in their own country. Therefore, the risk of being bitten by a rabid animal abroad and developing rabies after returning to Japan seems to be high. All medical staff should keep in mind that imported rabies cases can occur at any time. In addition, pre-exposure vaccination against rabies should be recommended to international travelers in order to ensure the preventative effect of PEP.

Key words Rabies · Vaccine · Post-exposure prophylaxis · Pre-exposure immunization · Lyssavirus · Hydrophobia

Introduction

Half a century has passed since rabies was eradicated in Japan. However, in November 2006, two cases of imported rabies occurred,[1,2] reminding us that rabies is not a disease which only existed in the past. From a world-wide point of view, countries that are free from rabies are the exception, and there are still many areas in the world where rabies continues to occur.[3] However, in regions where rabies is endemic, there are some areas where many patients die from rabies, and other areas where it rarely occurs. In Asian countries, hundreds or thousands of patients are killed by rabies every year. Considering the fact that the number of Japanese people traveling to Asian countries has recently increased, imported rabies cases are much more likely to occur than an invasion of rabid animals into Japan from these areas.

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Clinical features of rabies

Rabies is mainly a disease occurring in animals, and it is regarded as one of the most typical zoonoses. Rabies is known to have the following features.

(1) The incubation period of rabies is generally very long, ranging from 1 to 3 months (about 60% of cases) to more than 1 year (6%–7% of cases) (pathogenetic features).

(2) Almost 100% of patients who develop clinical rabies die, because the rabies virus causes fatal encephalomyelitis and no effective treatment has yet been developed. There are no laboratory tests to determine whether a person is infected with the rabies virus during the incubation period (clinical features).

(3) The host animals of the rabies virus differ among regions, even though almost every mammal is capable of contracting rabies. The main vectors are foxes in Europe and Canada, raccoons, skunks, and fruit-eating and insectivorous bats in the United States, dogs in Asia, mongooses, jackals, and dogs in Africa, and dogs and vampire bats in Latin America. There are two types of epizootic rabies, namely the urban type and the sylvatic type. The former type is where the rabies virus is principally transmitted among dogs, and the latter type is where the vectors are the wildlife, such as foxes, raccoons, and mongooses (epidemiological features).

Rabies is caused by the rabies virus, which is an enveloped, bullet-shaped, size 75 nm in diameter and 100–300 nm in length, single-stranded, minus-sense RNA virus.[3,4] It belongs to the genus *Lyssavirus* of the family *Rhabdoviridae*. Genus *Lyssavirus* includes some antigenically rabies-related viruses (rabies-related lyssaviruses). Most of these

are transmitted among bats, and some are reported to cause clinical rabies in humans. Genus *Lyssavirus* containing rabies virus is phylogenetically divided into two groups, phylogroups I and II. The former includes the rabies virus, Duvenhage virus, European bat virus types 1 and 2, and Australian bat virus; the latter includes Lagos bat virus and Mokola virus.[3,5]

History of rabies in Japan

It is not known for sure when rabies appeared in human society. It is presumed that man began living with dogs about 30000 years ago, so there is a possibility that human rabies has occurred since then. The oldest document mentioning rabies as a zoonosis is the law enacted around 2300 BC in Mesopotamia.[6] The law, the Eshunna Code, imposed a penalty on the owner of the dog when a bitten victim died from rabies. From this description, it is understood that the causal relationship had been clearly recognized; the person bitten by a rabid dog would have overt rabies and eventually die. Humans and dogs were increasing in number, and people started moving to new regions with their dogs. This movement seems to be the reason why rabies spread to various regions around the world.[7]

In Japan, a large epizootic of rabies was documented in Nagasaki Prefecture in 1732, and had spread to Oita Prefecture the following year.[8] Expanding along the main roads to Sanyodo and Tokaido, it eventually reached Edo in 1736, when Yoshimune Tokugawa was governing as the 8th Shogun. During this epizootic, many dogs, horses, foxes, raccoon dogs, etc. were killed. In 1736, Genjo Noro (1692–1761), one of the medical officials of the Tokugawa Shogunate, published *Kyoken-kosho-chiho*, which is the first textbook on the therapy for rabies.[9] In this book, he reported that the sickness would become serious after a certain period of time, and eventually 8 or 9 out of every 10 patients would die, even if the wound did not initially appear to be severe. Furthermore, he also wrote that the best first-aid treatment was to suck out the blood as quickly as possible and to apply moxa cautery to the wound. This textbook was republished in 1756, probably because the epizootic of rabies had not ceased.

Little information is available on epizootics of rabies in the latter part of the Edo Era. We can only assume that outbreaks of rabies occurred sporadically in those days based on the statistics of the number of rabid dogs during the Meiji Era (from 1868 to 1912), when 50–200 were recorded annually.

In 1895, an epizootic of rabies occurred in Nagasaki Prefecture. During this outbreak, Tomei Kurimoto, Chief Physician of Internal Medicine at the National Nagasaki Hospital, gave a rabies vaccination to people who were bitten by rabid dogs for the first time in Japan. He made an attenuated rabies vaccine by himself, following the method of the French scientist Louis Pasteur. He injected this vaccine into 25 patients who had been bitten by dogs, and as a result none of them died.[8]

Table 1. Numbers of rabid animals reported, regions where rabid animals occurred, and people bitten by rabid animals from 1911 to 1915 in Japan (from [10], with permission)

Year	No. of rabid animals	No. of regions	No. of people bitten
1911	570	10	904
1912	719	14	953
1913	856	18	1313
1914	1383	20	2602
1915	1424	24	3230

In the latter part of the Meiji Era, outbreaks of rabies gradually increased both in number and in scale. More and more outbreaks were reported in the Taisho Era (from 1912 to 1926), mainly in large cities (Table 1). Umeno and Doi, of the Kitasato Institute for Infectious Diseases, performed a mass rabies vaccination of dogs in Kanagawa Prefecture and Tokyo Prefecture in 1918 and 1919, respectively.[10] As a result, the numbers of both rabid dogs and people bitten by such dogs decreased significantly in both regions. However, both these numbers kept increasing outside of these regions. Owing to the widespread confusion after the great Kanto earthquake in 1923, reports of rabid dogs and human rabies cases exceeded 3000 and 100 per year, respectively, in the following 2 years. From that time, standard rabies control methods, such as compulsory vaccination of all family dogs and the elimination of stray dogs, were enforced all over Japan. Consequently, the number of rabid animals steadily decreased, reaching 15 or fewer during 1934–1943.[11–13]

However, the number began to increase again due to the social disorder after World War II. Seventy-six cases of human rabies and over 800 animal rabies cases had been reported in 1949 and 1950, respectively,[12,13] which led to the Rabies Prevention Law, enacted in 1950 (Fig. 1). This law requires owners to register, confine their dogs, and make sure that a rabies vaccination is administered. It was strictly enforced, in conjunction with the elimination of stray dogs.[13] The number of rabid animals decreased rapidly, and no rabies cases in either animals or humans have been reported since 1957, except for three cases of imported human rabies in 1970 and 2006.

In Japan, epizootics of rabies have historically been of the urban type, where the rabies virus was transmitted among dogs, and occasionally from dogs to humans, cats, or other domestic and wild animals. During such epizootics, foxes and raccoon dogs were also infected with the rabies virus. No transmitting circle of the virus has formed among the wildlife in Japan.

Epidemiology of human rabies

Japan was successful in eliminating rabies, although countries free from rabies are rather an exception worldwide and there are still many endemic areas. The numbers of human rabies cases differ within these areas, ranging from areas where hundreds or thousands of people die every year to regions where human rabies is very rare. The animals trans-

mitting rabies also differ among regions, so in order to diagnose and prevent rabies it is important that we understand the epidemiology of the disease.

There are no accurate statistical data of rabies deaths in every country. The number of human rabies cases is estimated to be 55000 per year worldwide, with 56% and 44% occurring in Asia and Africa, respectively.[3] It is believed that 84% of rabies cases break out in rural and poor regions. However, the numbers of rabies deaths do not correlate with the risk of contracting rabies, because it is possible to prevent a patient from dying of rabies by giving them post-exposure prophylaxis (PEP), even after they have been bitten by a rabid animal. Without PEP, the total number of human rabies deaths in Asia and Africa combined is estimated to be about 330000.[3]

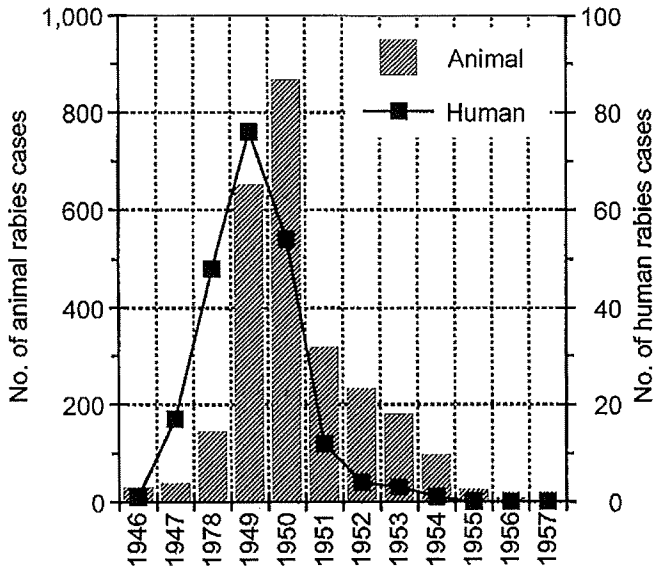

Fig. 1. Number of rabies cases reported in humans and animals in Japan after World War II. Just before World War II, the annual number of rabid animals reported was 15 or fewer because of the compulsory vaccination of family dogs and the elimination of stray dogs. However, rabies cases began to increase due to the social disorder after World War II, leading to 76 cases of human rabies in 1949 and more than 800 cases of animal rabies in 1950. In 1950, the Rabies Prevention Act was enacted. This requires dog owners to register and confine their dogs, and to vaccinate their dogs against rabies. This Act also strictly enforces the elimination of stray dogs. As a result, the number of rabid animals decreased rapidly, and no rabies case has been reported since 1957 in either humans or animals except for three imported human rabies cases. (The last human case was reported in 1954, and six rabid dogs were reported in 1956.)

In Asia, 95% of rabies is transmitted by dogs, whereas 3% is from cats. In the United States, foxes, skunks, and racoons are the host animals. In Latin America, dogs are the host animals of rabies. In addition to terrestrial animals, some species of bat transmit rabies in North and South America. In the United States, 17 out of 19 patients who contracted rabies domestically during 2000–2006 were infected by bats.[14] In cases of bat bites, it is assumed that patients often miss out on the opportunity to receive PEP because bite wounds from bats are too small to be noticed.[15]

Infection route of rabies

Humans usually contract rabies through bite wounds from rabid animals (bite exposure) because the rabies virus is highly concentrated in the saliva of infected animals. It can also be transmitted through nonbite exposure, although this rarely occurs. Airborne infections, such as inhaling an aerosol of infected animal brain tissue in virus laboratories, or of contaminated air in bat-inhabited caves, have been reported.[16–19] There is also one known case where a butcher became infected by skinning a cow that had died of an undiagnosed neurological disease.[20] Iatrogenic rabies cases have occurred in patients who received cornea, kidney, liver, or blood vessel graft transplantation from donors who had undiagnosed rabies (Table 2). To date, the only medically verified cases of human-to-human rabies transmission are the cases infected through organ transplantation from undiagnosed rabies patients.[21–29]

Table 2. Human rabies cases through organ transplantation

Case	Country	Year	Organ	Incubation period	Clinical diagnosis of donor	Reference number
1	USA	1979	Cornea	30 days	GBS	21
2	France	1980	Cornea	33 days	Encephalitis, myocarditis	22
3	Thailand	1981	Cornea	22 days	Not diagnosed	23
4	Thailand	1981	Cornea	31 days	Not diagnosed	23
5	India	1987	Cornea	2 days	Not described	24
6	India	1987	Cornea	257 days	Not described	24
7	Iran	1994	Cornea	26 days	Food poisoning	25
8	Iran	1994	Cornea	40 days	Food poisoning	25
9	USA	2004	Liver	20 days	SAH	26–28
10	USA	2004	Kidney	26 days	SAH	26–28
11	USA	2004	Kidney	26 days	SAH	26–28
12	USA	2004	Arterial fragment	25 days	SAH	26–28
13	Germany	2005	Lung	Not described	Not described	29
14	Germany	2005	Kidney	Not described	Not described	29
15	Germany	2005	Kiney/pancreas	Not described	Not described	29

GBS, Guillain–Barré syndrome; SAH, subarachnoid hemorrhage

Rabies through organ transplantation

In 1978, a 37-year-old American woman received a right corneal transplant from a 39-year-old lumberman who was presumed to have died from Guillain–Barre syndrome. Thirty days after the operation, she complained of right retro-orbital headache. In a few days, she noticed hypoesthesia on the right side of her face and difficulty in walking, and symptoms of dysphagia and dysarthria developed. After admission to hospital, she developed flaccid paralysis, became progressively obtunded, and eventually died on day 16 of hospitalization. A postmortem examination revealed rabies virus in the cornea, optic nerve, temporal lobe, and brain stem.[21]

In 1980, a 36-year-old man received a left corneal transplant from a 57-year-old woman who had died from encephalitis and myocarditis in France. Thirty-three days after the operation, he complained of left retro-orbital headache. Over the next 4 days he developed hypersalivation, pain and weakness in the legs, and pain on swallowing, and was hospitalized 41 days after the operation. He became comatose on day 3 of hospitalization and died on day 9. Rabies virus was isolated from the patient's brain tissue, and on histopathological examination numerous Negri bodies were found in the donor's brain.[22]

In 1981, a 41-year-old Thai woman received a corneal transplant from a boy who had died from an undiagnosed disease with mental confusion. A 25-year-old man also had a cornea transplant from the same donor. These recipients died 22 and 31 days after the operation, respectively. Rabies virus was isolated from the woman's brain tissue, and Negri bodies were found in the donor's brain tissue.[23]

In 1987, two Indian men received corneal grafts from a single donor. Nine days later, one of these recipients, a 62-year-old physician, reported redness, swelling, and intense pain in the operated eye. He died 14 days after the operation. The other recipient, a 48-year-old man, was advised to receive PEP. He received the first and second doses of rabies vaccine, but refused to take the remaining doses. He experienced dysphagia along with pain, redness, and swelling in the operated eye 257 days after the operation. Two days later, he developed hydrophobia. He died 5 days after the onset of the disease. The incubation period of the second man was more than 250 days, probably because he had received two doses of rabies vaccine.[24]

In 1994, a 40-year-old man received a corneal transplant from a 20-year-old man who had died from food poisoning in Iran. On the same day, another 35-year-old man received a cornea transplant from the same donor. The first patient reported nausea and paresthesia on his lips, and developed hydrophobia 26 days after the operation. He died within the next 24 h. The second patient was admitted to hospital with vomiting and poor general condition 40 days after the operation. He died the following day. Rabies virus was isolated from the brain tissue.[25]

In the United States, kidneys, liver, and an arterial segment were transplanted into four recipients from a common donor in 2004. All four recipients developed encephalitis within 30 days after transplantation, and died from rabies 7–23 days after the onset of neurological symptoms.[26–28]

In Germany, there was an announcement on February 16, 2005, that three out of six patients who had received organ transplantations from a common donor might have clinical rabies. The donor died after cardiac arrest and brain death in late 2004. Rabies was diagnosed in the donor and two of the recipients on the same day as the announcement and the next day, respectively.[29]

These cases indicate that organ transplants should not be carried out from donors who had died from encephalitis of unknown cause. At the same time, they also show that rabies is very difficult to diagnose intravitam.

Clinical course of human rabies

The clinical course of human rabies is divided into four phases: the incubation period, the prodromal phase, the acute neurological phase, and the coma phase.[30]

The incubation period for rabies varies from around 15 days to 1 year or even longer. In about 60% of all rabies patients, the incubation period is 1–3 months, but 6%–7% of patients exhibited an incubation period longer than 1 year. The longest incubation period reported was 7 years, and was documented for a girl who migrated from Laos to the United States.[31] She had been bitten by a stray dog in Laos 7 years before the onset of clinical rabies. In general, the incubation period is shorter when the bite is to the head rather than the extremities, and is also shorter in children than in adults. During the incubation period, the rabies virus propagates in the muscle cells around the port of entry and invades the peripheral nervous system. It then migrates centrally to the central nervous system, following the flow within the axoplasm of peripheral nerves at a velocity of 8–20 mm per day.[4] The symptoms of rabies first appear after the virus enters the central nervous system (prodromal phase).

In the prodromal phase, which lasts for 2–10 days, the patients complain of nonspecific symptoms such as malaise, fever, and anorexia. They may also complain of more specific local symptoms such as itchiness, pain, and paresthesia around the healed bite wound.

The acute neurological phase continues for 2–7 days. During this phase, patients will intermittently suffer from intense anxiety, emotional agitation, and confusion. At other times they may be calm, lucid, and cooperative toward the medical staff. About 60% of patients will develop severe pharyngeal and laryngeal muscle spasms when they attempt to drink, or even see, water (hydrophobia). Similar symptoms may also be induced when cool air blows on the face or chest (aerophobia). As a result, patients avoid drinking water, washing their hands, or feeling wind. The patient's condition gradually deteriorates. High fever, confusion, disorientation, paralysis, and general convulsions may occur, and the patient eventually falls into a coma.

In the coma phase, autonomic instability becomes extremely predominant, and hypotension, arrhythmia, and hypoventilation may develop. Most patients die shortly

after the onset of coma if no intensive supportive care is given.

No effective therapies for overt rabies have been established, so almost 100% of patients are destined to die. As of August 2007, there have been only six reports of patients recovering from overt rabies.[3]

Clinical and laboratory diagnosis of human rabies

A clinical diagnosis might be possible if the patient could describe the animal which had bitten them and in which rabies endemic area, and also if they showed typical symptoms such as hydrophobia or aerophobia. However, it is rarely possible to diagnose rabies clinically in Japan because the patient's history of animal bites is uncertain in most cases, and very few Japanese physicians have experienced clinical rabies.

An intravitam diagnosis of rabies could be made either by isolating the virus from saliva or cerebrospinal fluid, demonstrating a viral antigen in skin biopsy samples or corneal impression samples using the fluorescent antibody method, or detecting viral genes by reverse polymerase chain reaction (RT–PCR).[3,32] However, these laboratory methods are only useful after the virus has propagated into the brain tissue or disseminated to other parts of the body. They are not useful in the early stage of the disease, so it is practically impossible to diagnose rabies shortly after the onset.

Treatment of rabies patients

No medical treatment for clinical rabies has been established. Treatment is mainly aimed at minimizing the clinical signs and symptoms, and especially at reducing physical and psychological pain. Patients should be cared for in a private room with sufficient sedation. The intravenous administration of morphine is effective to relieve anxiety, agitation, hydrophobia, and aerophobia. Life-support measures should be avoided after rabies has been confirmed.[3] One case was reported of a patient who survived rabies after the use of heavy sedation in addition to antiviral medication,[33] but other clinicians were unsuccessful using the same method.[34] Before a patient is treated with these new therapies, the patient and their family should be informed of the possibility of severe neurological sequelae even if the patient did recover.[3]

Post-exposure prophylaxis (PEP)

Animal and human rabies still occurs in many Asian countries, although neither animal nor human rabies has been reported in Japan since 1957. Travelers in the endemic regions who are bitten by a possibly rabid animal need to receive PEP as early as possible. The World Health Organisation (WHO) recommends the following post-exposure treatment.[3] The bite wound should be thoroughly washed with soap and water. Next, as much human rabies immunoglobulin (HRIG, 20 IU/kg) or equine rabies immunoglobulin (ERIG, 40 IU/kg) as possible should be injected around the wound, and the remainder should be given intramuscularly. In addition, a tissue-culture-inactivated rabies vaccine should be administered on days 0, 3, 7, 14, and 30, and also on day 90 if necessary. PEP should be given to patients who request treatment even months after the bite, since an incubation period of longer than 12 months has been reported in 6%–7% of rabies cases.

The risk of contracting rabies will be higher when the patient is bitten on bare skin as opposed to through clothing, because the rabies virus is highly concentrated in the saliva. Moreover, when the face or the fingers are bitten, the incidence of rabies tends to be higher and the incubation period tends to be shorter than when the lower limbs are bitten.

Inactivated rabies vaccines marketed throughout the world are effective against lyssaviruses belonging to Phylogroup I, but are not effective against Phylogroup II.[3]

In Japan, a tissue-culture-inactivated rabies vaccine for human use (PCEC–K) is produced by a private manufacturer, the Chemo-Sero-Therapeutic Research Institute (Kaketsuken). However, neither HRIG nor ERIG is produced or imported in Japan, and furthermore, the Japanese Government has no stock of RIG. As RIG is not available in Japan, following the WHO recommendation for rabies PEP is not feasible in practice.

The PCEC-K vaccine is prepared from an attenuated rabies strain, HEP–Flury, which is grown in primary cultures of chick embryo cells. It is then inactivated with betapropiolactone, followed by concentration and purification.[35] Its antigen titer has not been officially announced. Researchers in Thailand reported that PCEC–K is less potent than rabies vaccines produced in France and Germany.[36]

PEP in our vaccine clinic

During 2000–2005, the number of patients visiting our vaccine clinic to receive PEP after being bitten by a supposed rabid animal abroad was 71–84 per year except in 2003, when the severe acute respiratory syndrome (SARS) suddenly occurred (Fig. 2). Among these patients, about 30% and 20% were between the ages of 20–24 years and 25–29 years, respectively. Eighty percent of the patients were bitten by animals in Asian countries, and in particular Thailand (40%). In detail, 81% of subjects were bitten by dogs, 11% by monkeys, and 4% by cats. About 55% of the patients attacked by animals overseas visited a local medical institute and received a rabies vaccine. The remaining 45% returned to Japan without receiving proper treatment abroad, and visited our clinic after being warned by their family members or friends of the possibility of rabies and the need for PEP.

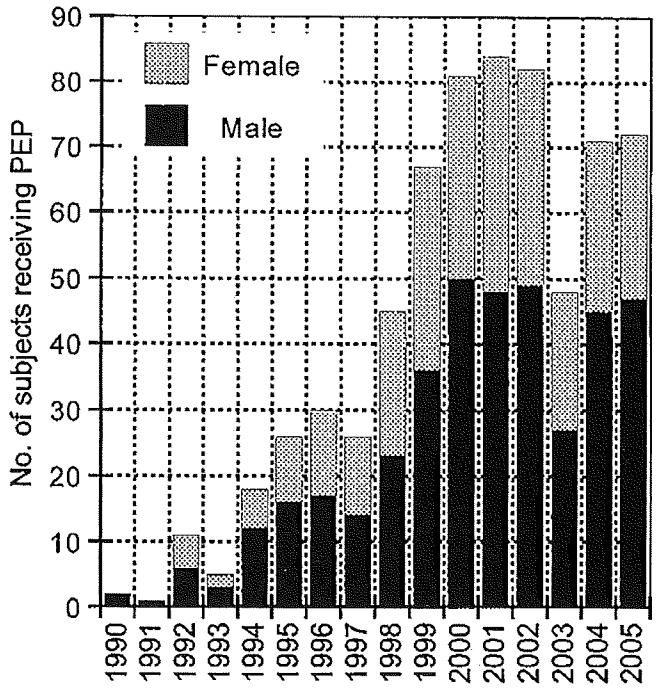

Fig. 2. The annual number of patients visiting our vaccine clinic to receive rabies post-exposure prophylaxis (PEP). The first overseas traveler bitten by a stray dog and requesting rabies PEP came to our vaccine clinic in 1990. The number of individuals receiving PEP annually in our clinic remained below 20 until 1994. However, it exceeded 20 in 1995, and continued to increase from 1997 to 2001. During this period, the annual number of Japanese people going abroad was increasing. However, we cannot explain the increase in the number of patients requesting rabies PEP simply by the rise in the number of Japanese international travelers. One speculation is that the need for rabies PEP has gradually been recognized among young Japanese adults attacked by suspected rabid animals abroad because nowadays they can easily obtain information through the Internet. On the other hand, not so many Japanese medical institutions have rabies vaccine in stock, which leads to a concentration of patients at the limited number of hospitals capable of providing rabies PEP. The decrease in the number of such patients in 2003 seems to have been caused by the outbreak of severe acute respiratory syndrome (SARS)

Imported rabies cases

In France, 19 cases of imported rabies have been reported since 1977. In England, 20 imported rabies cases were reported from 1946 to 2000. Since 2001, 2 cases have been reported in Germany, and one case each in France, England, and Taiwan.[37]

In Japan, three cases of imported rabies have been reported as of July 2007. The first case was a young adult who was bitten by a stray dog in Katmandu, Nepal, during a personal trip. About 1 month later, he complained of respiratory distress and died on the day of admission to hospital. He did not receive rabies PEP in either Nepal or Japan. Rabies was diagnosed based on the findings of a postmortem histological examination. The second and third cases were both men in their sixties.[1,2] During their long stay in the Philippines they were bitten by privately owned dogs, and they returned to Japan in November 2006 without having received rabies PEP. In both patients, the rabies virus was isolated from the saliva, and they were both diagnosed antemortem. By analyzing the gene, the rabies virus strains isolated were identified as the strain transmitted in the Philippines. In the third case, it was possible for medical staff to take the preventive measures recommended by the Center for Diseases Control and Prevention,[38] as the diagnosis was made shortly after the clinical symptoms appeared. This case was given similar treatment to the 15-year-old survival case,[31] but the treatment was unsuccessful.[1]

Pre-exposure immunization

Pre-exposure vaccination against rabies is a useful measure to prevent imported rabies. Pre-exposure immunization is recommended to people who are living in, or traveling to, high-risk regions. The WHO recommends a dose of tissue-culture rabies vaccine, with a potency of at least 2.5 IU per dose, to be given intramuscularly on days 0, 7, and 28.[3]

In Japan, pre-exposure immunization consists of two doses of PCEC–K given 30 days apart, and an additional dose given 6 months after the second dose.[35] Japanese travelers rarely plan their trip 6 months or more in advance, with the exception of some public employees. In many cases, the period available before leaving Japan is 2 months at the most. When they do not have enough time to complete the three doses, we recommend taking at least 2 doses. It is reasonably thought that patients who are bitten by a supposed rabid animal in an endemic area could efficiently produce antirabies antibody after receiving another two or three doses of the vaccine, and that they would then be protected against rabies without using RIG.

It is safer for people who are scheduled to be engaged in outdoor investigations or to handle animals to receive three doses of rabies vaccine, as recommended by the WHO, before leaving for rabies-endemic countries.

Conclusion

Half a century has passed since rabies was eradicated in Japan. However, countries free from rabies are exceptionally rare. Travel to Asian countries where many rabies victims still occur is easy, and only takes a few hours by airplane from Japan. Since Japanese travelers are rarely aware of rabies, they are at great risk of being bitten by a potentially rabid animal. Even if domestic human rabies cases no longer occur in Japan, imported rabies cases are always possible. Therefore, it is clearly important that no traveler ever carelessly puts out a hand to an animal in a rabies-endemic country. If a traveler is bitten by a suspected rabid animal in a rabies-endemic region, it is important that they receive rabies PEP in a local medical institution immediately. At the same time, pre-exposure vaccination against rabies should be recommended to international travelers in order to ensure the preventive effect of PEP. We should never forget that rabies is a preventable, but incurable, disease.

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