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

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].
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 *Toxocara 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 article 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 seroepidemiological 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 Japa 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 of 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.

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 (range, 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.

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. Allergic 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.

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.
[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 indefinite 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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