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▣ CASE REPORT

Trichinosis Caused by Ingestion of Raw Soft-Shelled Turtle Meat in Korea

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Abstract: Reptiles, unlike mammals, have been considered to be unsuitable hosts of *Trichinella* spp., though larvae have been detected in their muscles and human outbreaks related to their consumption have, in fact, occurred. Herein we report 2 Korean cases of trichinosis, possibly transmitted via consumption of reptile meat. Both patients suffered from myalgia, headache, and facial edema. Laboratory examinations revealed leukocytosis with eosinophilia (54% and 39%, respectively) and elevated creatinine phosphokinase. ELISA was performed under the suspicion of trichinosis, showing positivity at the 29th and 60th day post-infection. Since they had consumed raw soft-shelled turtle meat, turtle was strongly suggested to be an infection source of trichinosis in Korea next to the wild boar and badger.

Key words: *Trichinella* sp., trichinosis, soft-shelled turtle, Korea

INTRODUCTION

Trichinosis is a food-borne zoonotic disease caused by species of the nematode *Trichinella* spp. [1]. Pork remains the predominant source of human infection throughout the world, though other meats occasionally have been implicated as well [2]. Neither reptiles nor other cold-blooded vertebrates had been considered possible sources of human infection, until recently, when non-encapsulated *Trichinella* sp. larvae were detected in the muscle tissue of crocodiles from Zimbabwe, and subsequently grouped under the classification of *Trichinella zimbabwensis* [3]. Later, larvae of *Trichinella papuae* were detected in saltwater crocodiles from Papua New Guinea [4]. The zoonotic potential of these crocodiles was confirmed by successful experimental infection of laboratory rats, baboons, and domestic pigs [5]. The first human trichinosis outbreak resulting from ingestion of raw soft-shelled turtle meat occurred in Taiwan [6]. Korea has been known to be an endemic region of trichinosis since the first trichinosis patients were identified in 1997 and thereafter 5 additional outbreaks have been recorded [7-12]. We report herein a new outbreak of trichinosis involving 2 cases in Korea, which were caused by ingestion of raw soft-shelled turtle meat.

CASE RECORD

A 30-year-old man, residing in Cheongju-si, Chungcheongbuk-do, was admitted to the Department of Internal Medicine, Cheongju St. Mary's Hospital, Cheongju, Korea on 5 September, 2012, complaining of myalgia. He had no relevant medical history, though 3 weeks previously, facial edema, headache, and poor oral intake was developed, and 10 days before his admittance, myalgia and muscle tremor were added to that list. His body temperature was 36.5℃, and C-reactive protein (CRP) was within normal limits. A complete blood count (CBC) showed marked (54%) eosinophilia along with 16,090/μl leukocytosis. Biochemical examinations showed that creatinine phosphokinase (CPK) level was elevated to 349 IU/L (normal range; 24-172), and lactic dehydrogenase (LDH) was 314 IU/L (140-271). Chest radiography and urinalysis showed nothing abnormal. He had consumed raw soft-shelled turtle meat with 6 of his colleagues 4 weeks previously at a restaurant in Jincheon-gun, Chungcheongbuk-do, and all but one among their group had been suffering from similar symptoms.

On 6 September 2012, ELISA was performed under suspicion of trichinosis in the Department of Parasitology, Seoul

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National University College of Medicine. The production of crude antigen of *Trichinella spiralis* was as follows. Routinely maintained *T. spiralis* larvae in the laboratory were liberated from a mouse by the artificial digestion method. Then, the larvae were chopped and homogenized in ice, centrifuged at 15,000 rpm for 1 hr at 4℃, and the supernatant was used as the crude antigen. The protein concentration of *T. spiralis* crude antigen was 0.974 mg/ml. The positive control was provided by the sera of the 5th outbreak patients [11], and anti-human IgG horseradish peroxidase diluted in 1:24,000 (Cappel Inc., Costa Mesa, California, USA) was used as the secondary antibody. Other procedures of ELISA were the same as those of *Clonorchis sinensis* ELISA [13]. By ELISA, the serum of the present case showed positivity against *T. spiralis* larval antigen, 0.293 in optical density (OD), whereas negative controls were 0.0 in OD and positive controls were 0.646 in OD. He was treated with albendazole for 2 weeks, over the course of which his myalgia and muscle tremor symptoms were gradually improved.

The second patient was a 38-year-old man, residing in Eumseong-gun, Chungcheongbuk-do. His chief complaints and clinical course were the same as those of the first patient. The CBC revealed 39% eosinophilia and 13,360/μl leukocytosis, and CRP was slightly elevated to 5.9 mg/L (normal range: <5.0). Biochemical examinations revealed relatively high CPK (364 IU/L) and LDH (304 IU/L) values. ELISA performed on the 29th day post-infection (PI), was negative against *T. spiralis* larval antigen (0.157 in OD in the patient vs 0.0 in OD in negative controls and 0.646 in OD in positive controls), but showed positivity on the 60th day PI (0.429 in OD; 0.0 in OD in negative controls and 0.710 in OD in positive controls). As in the case of the first patient, he was treated with albendazole for 2 weeks, and by October 2, almost all of the clinical signs of trichinosis disappeared, from this second patient and also from the first patient, with the exception of minor muscle tremor.

DISCUSSION

The outbreak of trichinosis reported herein is the 7th in Korea (Table 1). Only 2 of 6 patients could be examined; even so, the present cases are noteworthy for their extraordinary nature. Although the first outbreak had been traced to ingestion of badger meat, the next 5 were due to consumption of raw wild boars' flesh, the leftovers of which were found to contain the *Trichinella* sp. larva [9-12]. The 7th outbreak (2 cases) is the first to be implicated with raw soft-shelled turtle meat.

Human trichinosis consequent upon ingestion of reptile meat is rare worldwide. The lizard, *Varanus nebulosus*, was the infection source in the first (Thai outbreak) and second outbreak (Taiwanese outbreak, in which 8 people were infected) having been attributed to consumption of raw meat, blood, and eggs of the soft-shelled turtle, *Pelodiscus sinensis* [6,14]. The present trichinosis outbreak, then, is only the 3rd implicating with reptile meat. Interestingly, the turtle had been considered to be an unsuitable host of *Trichinella* spp., since *T. papuae* and *T. zimbabwensis*, for 2 instances, had shown only a very low reproductive capacity therein [2]. Hence, the present report has the special significance. Clearly, a comprehensive survey estimating the Korean prevalence of trichinosis in soft-shelled turtles, as in wild boars, is urgently required.

Although the present patients manifested the same clinical courses as previous trichinosis patients who had consumed mammal's meat, the causative agent was supposed to have been *Trichinella* sp. other than *T. spiralis*. Among the 8 species and 3 genotypes, only *T. papuae* and *T. zimbabwensis* can infect reptiles [15]. Since neither *T. papuae* nor *T. zimbabwensis* larvae induce capsule formation during the muscle phase of infection [16], muscle biopsy can be useful in *Trichinella* spp. identification. Muscle biopsy, unfortunately, due to its invasive nature, cannot be applied in all cases. Correct diagnosis therefore might depend on other methods, for example, species-specific antibody responses. As regards the Taiwanese outbreak, it was

Table 1. Outbreaks of trichinosis occurred in Korea

No.	Date	No. of Patients	Infection source	Locality	Diagnosis [Reference]
1	1997. 12	4	Badger	Geochang, Gyeongsangnam-do	Muscle biopsy, Ab test [7,8]
2	2001. 2	5	Wild boar	Inje-gun, Gangwon-do	Muscle biopsy, ELISA
3	2002. 2	4	Wild boar	Gangwon-do	Muscle biopsy, PCR [9]
4	2003. 3	13	Wild boar	Inje-gun, Gengwon-do	Muscle biopsy, ELISA [10]
5	2010. 11	8	Wild boar	Yanggu-gun, Gangwon-do	ELISA (+) in 5 patients [11]
6	2010. 12	12	Wild boar	Gangwon-do	ELISA (+) in 3 patients [12]
7	2012. 9	6	Soft-shelled turtle	Jincheon-gun, Chungcheongbuk-do	ELISA (+) in 2 patients [Current case]

suggested that the patients were likely to have been infected with *T. papuae*, since their convalescent-phase sera reacted more strongly to that protein [6]. Although species identification was unavailable in the present outbreak, sera-based diagnosis should be applied to all future cases of trichinosis to determine the causative species of *Trichinella*.

As the antibody titers have changed during the course of infection, the point of blood-gathering should be important. In experimentally infected goats with *T. spiralis*, ELISA showed the first increments in OD at week 2 PI, reached their peak at week 4 PI, and remained elevated from that day until week 10 PI [17]. Hence, ELISA can deliver false-negative results during the early stage of infection. However, the infection dosage also influences the antibody dynamics. In the above experiment, the inoculation dosage was 10,000 larvae per goat, but it should be varied in human patients. In addition, immune dynamics could be varied according to each patient. Hence, positivity usually takes at least 34 days after infection, but negativity is possible even at day 42 PI [11].

In the present cases, the ODs of the patients were lower than those of the positive control sera. This could be explained by that the sera-collection time was different between the patients of the 5th outbreak and the current one, although it could be somewhat varied according to each patient. In the present cases, the point of the first patient (day 29 PI) was earlier than that of the 5th outbreak (day 34 PI) and that of the 2nd one (day 57 PI) which was too late. Thus, in the 5th outbreak, only 5 of 8 clinically diagnosed patients were confirmed by ELISA, and in the 6th outbreak, only 2 of 12 patients were positive. Given the ELISA's low sensitivity, not to mention the general lack of knowledge of trichinosis among physicians, it can be assumed that the disease is underreported in Korea. Development of alternative, high-sensitivity diagnostic tools needs to be priority of the present and future research.

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