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## Research Note

# Clinical and etiological study of a small familiar outbreak of trichinellosis in Tibet, China

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## Summary

The diagnosis of trichinellosis is rather difficult because fever, myalgia and eosinophilia are nonspecific, and diagnosis may be delayed. We describe a small familiar outbreak of trichinellosis occurred in early 2009 in Tibet, southwestern China, due to consumption of raw pork. The patients with trichinellosis were diagnosed by muscle biopsy and serological tests (ELISA and IFA) early at 12 days after onset of disease. Both of the pre-encapsulated and encapsulated *Trichinella* larvae collected from biopsy muscles and residual pork were identified as *Trichinella spiralis* by multiplex PCR. This is the first species identification of *Trichinella* isolates from the biopsy muscles of patients with trichinellosis in China.

**Keywords:** trichinellosis; outbreaks; early diagnosis; species identification; Tibet; China

## Introduction

Trichinellosis is caused by the tissue-dwelling nematode *Trichinella*. Nine species of the genus *Trichinella* have been identified in animal hosts, but *Trichinella spiralis*, often found in domestic pigs, is the most common etiological agent of human trichinellosis (Pozio 2007; Murrell & Pozio, 2011). Humans acquire the disease by ingesting raw or insufficiently-cooked meat of pigs or other animals containing the *Trichinella* larvae. Following ingestion of infested meat, the *Trichinella* larvae are liberated from their capsules to invade the upper small intestine and mature into adult worms. The hallmark of the intestinal phase, usually lasting 1 week, is a non-specific gastroenteritis. Diarrhea, abdominal pain, nausea, and vomiting can occur. In 2 – 3 weeks, fertilized females produce ~1500 larvae, which migrate via blood and the lymphatic system to invade and encapsulate in skeletal muscles (muscular or par-enteral phase). The muscular phase is associated with an inflammatory and allergic response to muscle invasion by the migrating larvae. Fever, eyelid or facial edema, myalgia, and eosinophilia are the most prominent manifestations (Wang *et al.*, 1998; 2006). Trichinellosis is cosmopolitan, and has been documented in 55 (27.8 %) countries around the world (Pozio, 2007). In China, the first case with trichinellosis occurred in 1964 in Tibet. From then

until 2009, 580 outbreaks of trichinellosis, consisting of 25219 cases and 251 deaths, were recorded in 12 out of 34 Provinces/Autonomous Regions/Municipals of China (Cui *et al.*, 2011). Although the incidence of trichinellosis in China has decreased in recent years, outbreaks of trichinellosis still occurred in southwestern China, where ethnic groups usually eat raw meat. So, trichinellosis is an important foodborne parasitic disease with health, social, and economic impacts in China. However, the etiological agent of human trichinellosis in China was not clear; it was assumed to be *Trichinella spiralis*. All *Trichinella* larvae in muscle biopsy of patients have not been identified at the species level although *Trichinella* isolates from domestic pigs and dogs were identified as *T. spiralis* and *T. nativa*, respectively (Wang *et al.*, 2007, 2012).

## Clinical study

A small familiar outbreak of trichinellosis occurred in early 2009 in Zhula village, Gongbujiangda County, Nyingchi District of Tibet, southwestern China. The two patients were husband (33-years) and wife (32 years). One outdoor-raised pig was slaughtered at home without veterinary inspection of the meat, raw pork were consumed by the 2 patients in February 1, 2009.

One day later, our 2 patients complained of abdominal pain and

diarrhea. Two to 7 days later, they developed fever, periorbital and facial edema, edema of lower limbs and general myalgia. Their 2 daughters who ate cooked pork did not have any symptoms. Both patients were hospitalized locally, treated with penicillin for suspected infectious diseases, but they did not improve, and therefore they were referred to the Nyingchi People's Hospital. On hospital admission, their main manifestations were fever, myalgia, and edema of lower limbs. They had no splinter hemorrhages, hepatosplenomegaly, lymphadenopathy, and skin rash. Laboratory examinations revealed leukocytosis (9,100 cells/ $\mu$ L and 9,800 cells/ $\mu$ L) and eosinophilia (17.6 %, 1,600 cells/ $\mu$ L and 12.2 %, 1,200 cells/ $\mu$ L, respectively).

Because the symptoms and eosinophilia were highly suggestive of trichinellosis, the information was reported to the local Centers for Disease Control. Blood samples were taken and muscle biopsy was performed at 13 days after eating raw pork. About 2 g of gastrocnemius muscles was removed from each of the suspected patients. Serum and muscle samples were mailed to the Department of Parasitology, Medical College, Zhengzhou University, China. The specific anti-*Trichinella* antibodies in serum samples of

two patients were detected by both enzyme linked immunosorbant assay (ELISA) using excretory-secretory (ES) antigens of *T. spiralis* muscle larvae (Jing *et al.*, 2014) and immunofluorescence test (IFT) using the frozen section antigen of the purified *T. spiralis* muscle larvae (Cui *et al.*, 2014). The specific antibodies against other tissue-dwelling helminthes (*Paragonimus skrjabini*, *Clonorchis sinensis*, *Schistosoma japonicum*, and *Spirometra masoni*) were also assayed by IFA. The two serum samples had negative results for other helminthes.

The biopsy gastrocnemius muscles were examined by the compression method using two slide glasses. The pre-encapsulated *Trichinella* larvae were found in two patients and 192 and 196 pre-encapsulated larvae per gram (lpg) of muscles were collected respectively. In section specimen of the biopsy muscle, the transverse and longitudinal sections of the pre-encapsulated *Trichinella* larvae were also found (Fig. 1).

The two patients were treated with albendazole (30 mg/kg.d in two doses for 14 days). Both patients cleared their fever within 2 day following treatment. After one week of treatment, the male patient had total WBC and eosinophil counts of 5,100 cells/ $\mu$ L and

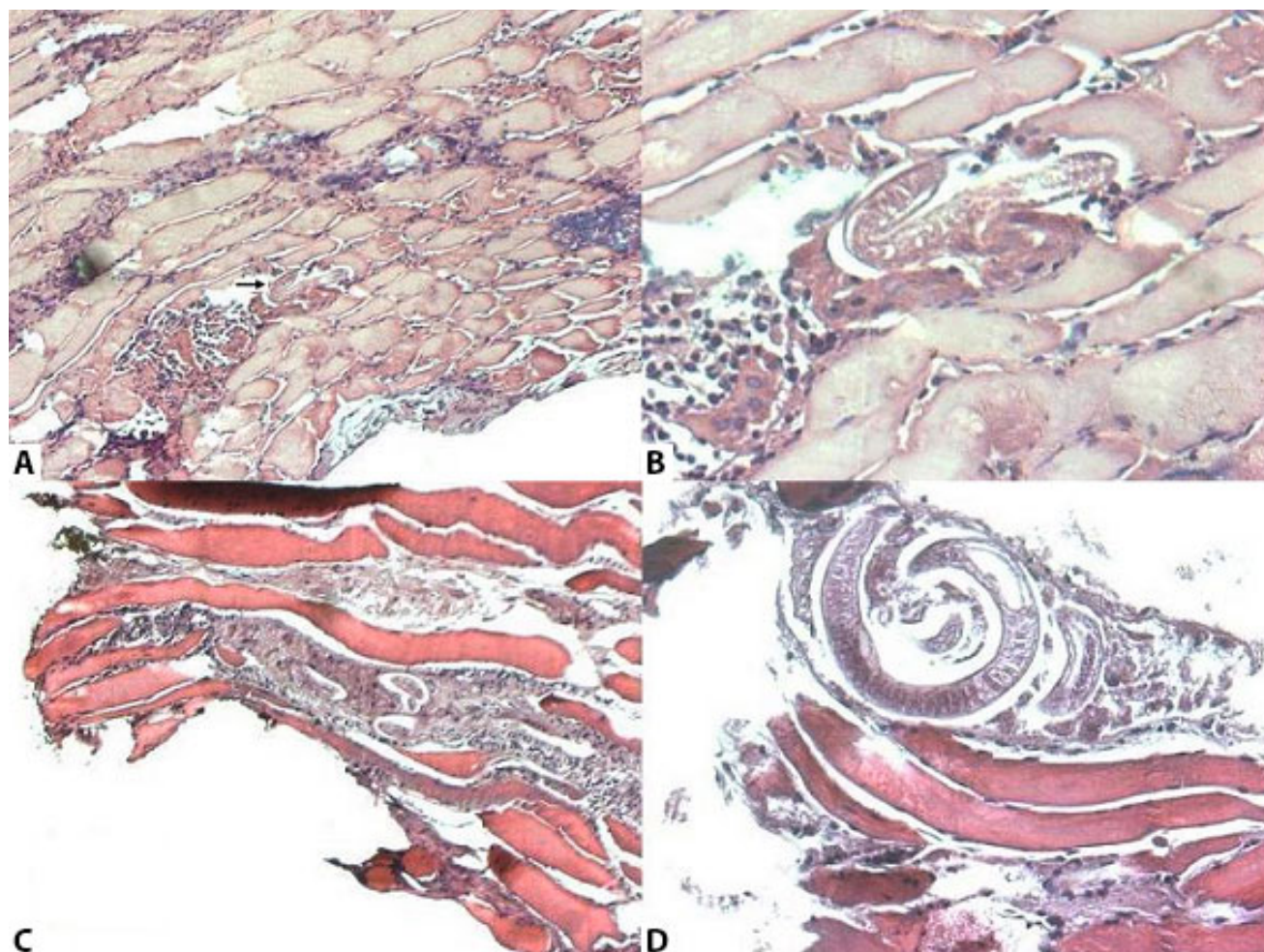


Fig. 1. A section of the pre-encapsulated *Trichinella* larva in the skeletal muscle fibers from case 1 (Figs. 1A and 1B) and case 2 (Figs. 1C and 1D). **A** (magnification,  $\times 200$ ): the longitudinal section of the *Trichinella* larva in the skeletal muscle fiber (arrow head), the muscle fibers have lost their striations because of muscle degeneration during delivery. **B**: The high-powered view (magnification,  $\times 400$ ) of a longitudinal section of the coiled *Trichinella* larvae, and there is a marked infiltrate of inflammatory cells. The muscle fiber parasitized by a larva is enlarged. **C** (magnification,  $\times 200$ ): The transverse sections of the *Trichinella* larvae were found in skeletal muscles, without an obvious surrounding collagen capsule. **D**: The high-powered view (magnification,  $\times 400$ ) of a longitudinal section of the coiled *Trichinella* larvae. The structure of the larva is well preserved, suggesting that it is alive.

400 cells/ $\mu$ L, and the female patient had total WBC and eosinophil counts of 7,300 cells/ $\mu$ L and 300 cells/ $\mu$ L. By the time of hospital discharge (one week after treatment), both patients were symptom-free with normal eosinophil counts, and there have been no sequelae.

### Etiological study

Raw pork samples of the residual hind leg of pig from the patient's family were also collected, and examined by the compression method. The encapsulated *Trichinella* larvae were found. These pork samples were subsequently examined by artificial digestion method, and the motile coiled larvae were found with an intensity of infection of 12 lpg of pork.

The *Trichinella* larvae in biopsy muscles of the two patients and in residual pork were respectively collected by digestion method (Li *et al.*, 2010). The larvae from both biopsy muscles and pork were identified as *Trichinella spiralis* by multiplex PCR (Fig. 2), as previously described (Zarlenga *et al.*, 1999).

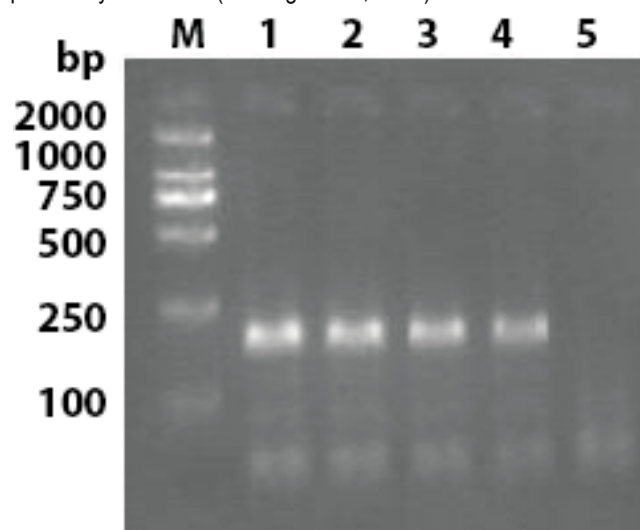


Fig. 2. Agarose gel separation of multiplex PCR products using DNA of *Trichinella* isolate from the patient's biopsy muscles and residual pork  
M – molecular weight markers; 1 – *Trichinella* isolate from case 1;  
2 – *Trichinella* isolate from case 2; 3 – *Trichinella* isolate from residual pork;  
4 – *T. spiralis* isolate (ISS534) control; 5 – negative control

### Discussion

Trichinellosis may mimic many clinical syndromes and is often misdiagnosed. History of eating raw or insufficiently cooked meat, a “gastroenteritis” or “flu-like illness” that followed ingestion of the suspect meat by 2 – 3 days gives a clue to the diagnosis. If two or more persons in the same household or the same community have fever, periorbital or facial edema and myalgia, trichinellosis can be suspected.

The short incubation periods in our patients may be related with high larval burdens. Eosinophilia has been observed in the majority of patients with trichinellosis. Eosinophilia appears early, before the development of general manifestations and it obviously increases at the second week of infection (Wang *et al.*, 1998). Absolute eosinophil counts vary (as high 19,000 eosinophils/ $\mu$ L) and correlate with degree of myalgia. The differential diagnosis of fever

and myalgia is very wide, but periorbital or facial edema with eosinophilia could narrow the diagnosis. The combination of eosinophilia and positive specific anti-*Trichinella* antibodies can establish the diagnosis of trichinellosis in most cases with trichinellosis.

The ELISA test is the most commonly used serological method for diagnosis of trichinellosis recommended by International Commission on Trichinellosis (ICT) (Gamble *et al.*, 2004). The use of the ES antigens of *T. spiralis* muscle larvae increases the test specificity and allows detection of anti-*Trichinella* antibodies as early as 2 weeks after infection. Sometimes the serodiagnosis is negative during the first days of the muscular phase, the combination use of ELISA and IFA tests can make the diagnosis earlier. Other parasitic diseases which manifest fever and eosinophilia in China include acute paragonimiasis, clonorchiasis, schistosomiasis, and sparganosis, a few of patients with trichinellosis might have detectable IgG antibodies cross-reacted with these parasites by ELISA or IFT test that yield false-positive results, which may lead to misinterpretation (Cui *et al.*, 2015).

The definitive diagnosis of trichinellosis can be made by finding larvae in biopsy skeletal muscles (gastrocnemius or deltoid muscles). Although some of biopsy muscles should be cut routinely for histopathological studies, a part must provide fresh and uncut for a direct microscopic examination (trichinelloscopy). Trichinelloscopy and especially digestion method are of great use in diagnosis because they detect the complete *Trichinella* larvae, defines the intensity of infection (i.e., the number of larvae per gram of examined muscles) and allows the collection of individual larvae, which can then be used to identify the parasite at the level of species (Dupouy-Camet *et al.*, 2002). Although the coiled larvae in collagen capsule are easy to find 4 weeks after infection, they may still be small, pre-encapsulated, and be easily omitted during the early phase of infection as they were seen in our patients.

Albendazole is the drug of choice for treatment of trichinellosis, it acts against the adult worms in intestines, migrating larvae, and pre-encapsulated larvae in muscles. During 2 – 3 days after treatment with albendazole, fever disappeared and myalgia was relieved in majority of patients (Wang *et al.*, 1998). But, albendazole is ineffective against the encapsulated larvae in the later phase of disease. The later the treatment is prescribed, the higher the probability that the patients will harbor viable larvae in their muscles for years, with possible persistent myalgia (Pozio *et al.*, 2001). Corticosteroids can be used for severe patients, but it must always be used in combination with anthelmintics and never alone, since it could prolong the intestinal phase of infection and increase the larval burden by delaying the expulsion of worms from the intestine (Dupouy-Camet *et al.*, 2002). Our two patients responded to albendazole and were cured without corticosteroids.

At present, outbreaks of trichinellosis continue to occur in mountainous areas of southwestern China, where pigs are raised in backyards under poor hygienic condition or in open areas where pigs are pastured freely and feed on raw waste products or animal carcasses. The local minor ethnic groups slaughtered the self-raised pigs at home without veterinary inspection (Cui *et al.*, 2006; Cui & Wang, 2011). In addition, the ethnic groups enjoy eating raw meat, such as raw skin, chop raw, cross bridge rice line (raw pork mixed with noodles and scalded for one to three minutes), and



acidic meat (fermented raw pork). The *Trichinella* larvae in biopsy muscles from our patients and residual pork were identified by multiplex PCR as *T. spiralis*. This is the first species identification of *Trichinella* larvae from the patients' biopsy muscles in China. Recently, outbreaks of trichinellosis occurred in the neighboring countries (Laos and Vietnam) (Barennes *et al.*, 2008; Taylor *et al.*, 2009), the major source of infection is due to local inhabitants eating raw pork contained *T. spiralis* larvae. These data indicate that in southwestern China and southeast countries, the main aetiological agent of human trichinellosis is *T. spiralis*, and pigs play an important role in the maintenance of the domestic cycle of *T. spiralis* (Conlan *et al.*, 2014). As an important public health concern, outbreaks of trichinellosis reinforce that the pig-rearing practices must be improved, mandatory inspection of pork and educational program further strengthened in southwestern China. Finally, this paper reports the continuing occurrence of trichinellosis and confirms that *T. spiralis* is the etiological agent of human trichinellosis in southwestern China. It also emphasizes the importance of pre-encapsulated *Trichinella* larvae in biopsy muscles for early diagnosis.

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