Experimental Observation on The Dynamic Changes of
*Trichinella Spiralis* in Host

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**Abstract. Objective:** To observe the developmental dynamics of *Trichinella spiralis* in the host. **Methods:** Ten female Kunming mice were involved in the experiments, each of which was infected with 100 *Trichinella spiralis* larvae. Two mice were respectively killed after the infection of 60 days, 120 days, 180 days, 300 days and 400 days. The cysts of *Trichinella spiralis* were detected with staining method. **Results:** After the infection of 60 days, the capsules were fully mature; 120 days later, inner layer began to appear transparent and inner layer were completely transparent up to 400 days. **Conclusion:** The longer the infection time, the fuller the capsule developed, that it is, the development trend was the shape from the spindle to the ovoid to the circular, and the inner layer of transparency gradually increased.

**Introduction**

*Trichinella spiralis* is also called trichina which can cause the trichinosis. It is one of the important human and zoonotic parasitic diseases, which was distributed worldwide. It was reported that trichinosis occurred in 121 cities and counties of 15 provinces or autonomous regions and cities in the mainland of China [1]. People infected with trichomonas through eating meat infected with the encysted larvae, around which the formation of capsules appeared after people were infected for 4 weeks [2]. The main pathogenesis of *Trichinella spiralis* is larvae, the development stages of which can be divided into the invasive period, larval transition period and capsule formation stage. After the formation of capsules, it is significant to investigate the changes of capsule shape and inter-capsule changes. In this study, the mice were infected with *Trichinella spiralis* larvae, and the dynamic changes of the larvae were observed in mice. The purpose of our experiments is to provide the experimental data for the prevention, diagnosis and treatment of trichinosis.

**Materials and Methods**

**Source of Muscle Larvae and Imago of Trichinella Spiralis**

The imago of *Trichinella spiralis* are introduced from CDC of Henan province, preserved and sub cultured in mice. Before the experiments, the muscle larvae were transferred to mice for spare. Each mouse was infected with 100 *Trichinella spiralis* larvae orally. 35 days post-infection, mice were sacrificed by breaking the neck and the pure muscle larvae are collected through artificial digestion method.
Animal Infection

The female Kunming mice with 60 days age weighted 22-26g were purchased from the experimental animal center of Zhengzhou University. The total of ten mice were infected with 100 *Trichinella spiralis* larvae orally, two of which were respectively killed after 60 days, 120 days, 180 days, 300 days and 400 days.

Collection of Specimens

The mice were killed by breaking neck. The complete diaphragm and part of abdominal muscle were cut into small pieces and pressed between two slides, tied with the thread at both ends.

Cysts Detection

After the samples were made into slices and stained, the samples were examined through the microscope and the images were collected under microscope.

Staining Method

Alum carmine staining was used in the experiment. Fixative solution and staining solution are described in the literature 3 in detail [3].

**Fixation:** The slices were placed in a fixed solution for 48 h; after unbinding, rinsed with water for 5 min. **Dyeing and separation:** the fixed slices were placed in the dyeing solution for 10 hours; the slices were taken off and separated for 2 min in 2% color separation solution of hydrochloric acidic alcohol. **Dehydration, transparent and seal:** After separation, the slices were dehydrated in 50%, 70%, 80%, 95%, 100% ethanol for 30 minutes in turn. The mixture of absolute ethyl alcohol and xylene (Proportion: 1:1) was used to conduct the transparency for 30 minutes, while pure xylene for 30 seconds. Two drops of neutral gum were dripped on the slide, the slide prepared was put into, covered with glass slides, and dried in the air at room temperature.

Results

The Slices Derived from The Mice Infected with Muscle Larvae for 60 days

**Microscopic observation:** the cysts show spindle-shaped with clear structure and thin wall, and have clear boundaries from the surrounding muscle fibers; the cysts show two layers, the inner layer (ie deep layer) was thicker, encapsulated larvae tightly which was spirally curled, the outside layer (ie, shallow layer) was present at both ends of the cysts obviously, there is a clear boundary between the inner and outer layers (Fig. 1 A)

The Slices Derived from The Mice Infected with Muscle Larvae for 120 days

**Microscopic observation:** The cysts are oval and have clear structure. The inner edges of the cysts are transparent. The outer layer (ie, the shallow layer) is thicker at both ends and thinner on both sides. (Fig. 1 B)

The Slices Derived from The Mice Infected with Muscle Larvae for 180 days

**Microscopic observation:** The cysts are oval, and have clear structure and thin wall. The inner edges of the cysts are transparent. The outer layer (ie, the shallow layer) is thicker at both ends. (Fig. 1 C)
The Slices Derived from The Mice Infected with Muscle Larvae for 300 days

**Microscopic observation:** The cysts are circular, and have clear structure and thin wall. The inner edges of the cysts are transparent and thicker. The outer layer (i.e., shallow layer) is around the inner layer obviously. (Fig. 1 D)

The Slices Derived from The Mice Infected with Muscle Larvae for 400 days

**Microscopic observation** (Fig. 1 E): The cysts are circular, and have clear structure and thin wall. The inner edges of the cysts are transparent and thicker. The outer layer (i.e., the shallow layer) surrounding the inner layer is uniformly thickened.

**Microscopic observation** (Fig. 1 F): The cysts are oval and the wall of the cysts is not clear with the fuzzy boundary between muscle fibers. The inner layer of the cysts (i.e., the deep layer) has not seen the deep dye area. However, the completely transparent edge of the inner layer can be identified. The larvae are curled up in an inner layer that was transparent. Around the inner layer of the outer layer (i.e., shallow layer) uniform thickening, inner and outer layer boundaries are not obvious. The outer layer (i.e., the shallow layer) surrounding the inner layer is uniformly thickened. The inner and outer layer boundaries are not obvious.

![Figure 1. Stained specimens after Trichinella infection (400 ×).](image)

A: The cysts derived from the mice infected with muscle larvae for 60 days
B: The cysts derived from the mice infected with muscle larvae for 120 days
C: The cysts derived from the mice infected with muscle larvae for 180 days
D: The cysts derived from the mice infected with muscle larvae for 300 days
E: The cysts derived from the mice infected with muscle larvae for 400 days
F: The cysts derived from the mice infected with muscle larvae for 400 days
Discussion

The results of the study indicate that when the mice are infected with trichinella spiralis for 60 d, the cysts are fully mature [4], the cyst wall is complete, the cyst is divided into two layers, the inner layer (i.e., deep layer) becomes thicker, which tightly wrap larvae, outer layer(i.e., shallow layer) is within the capsule at both ends, both inside and outside layer boundary is obvious; when the mice are infected with trichinella spiralis for 120 d, the shallow layer in the cyst increases, the deep layer slightly narrows, and some area of deep layer starts to become transparent; when the mice are infected with trichinella spiralis for 180 d, the deep layer of the specimens of trichinella spiralis infection in mice become more transparent; when the mice are infected with trichinella spiralis for 400 d, some the inner Layer of cyst is completely transparent.

The results of the study suggest that with the increase of infection time, the morphology of cysts and intracystic larvae have obviously changed. Firstly, the inner layer of the cysts (ie, the deep layer) is changed from the thick to shrink gradually, after being infection for 120 days, the inner layer begins to be transparent, the occurrence of transparency may extend evenly inwardly from the outer edge of the inner layer, or may gradually expand from a small extent of the outer edge of the inner layer until the inner layer is completely transparent. Secondly after being infected for 60 days, the larvae is wrapped in the inner layer, and basically the development is completely mature, no significant changes occurs continuously, no influence occurs with the time passes by. Thirdly, the change of outside layer of the cysts (ie, the shallow layer) occurs from the thinner to larger gradually. Fourthly the change of the morphology of cysts also occurs from the spindle to the oval and circular.

In summary, the longer the infection time, the fuller the capsule developed, that it is, the development trend was the shape from the spindle to the ovoid to the circular, and the inner layer of transparency gradually increased.

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References


