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Short communication

Tolerance to low temperatures of *Toxocara cati* larvae in chicken muscle tissue

Kensuke Taira[a], Yasuhide Saitoh[a,*], Natsuki Okada[a], Hiromu Sugiyama[b], Christian M.O. Kapel[c]

[a] *Laboratory for Parasitology, Department of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan*
[b] *Department of Parasitology, National Institute of Infectious Disease, 1-23-1 Toyama, Shinjyuku, Tokyo 162-8640, Japan*
[c] *Section for Zoology, Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark*

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ABSTRACT

Infectivity of *Toxocara cati* larvae in muscle tissue of chickens after storage at 4 °C and −25 °C was assessed in a mouse bioassay to provide information on the risk of meat-borne toxocarosis. Muscle tissue samples of 30-day old *T. cati* infections were stored at 4 °C for 14 and 28 days and at −25 °C for 12, 24 and 48 h, whereafter, larvae were released by digestion. For each experimental group, the released larvae were inoculated in six mice. After 15 days, mice were euthanized and larval burden was assessed by digestion. In the control group (no storage of the infected chicken meat), 47.9% of the inoculated larvae established in mice, whereas storage of meat at 4 °C for 14 days or 28 days reduced the recovery to 24.1% or 3.3%, respectively. Muscle larvae exposed to −25 °C for 12, 24 or 48 h did not establish in the mice. The observation that larvae retain infective after refrigeration at exposure in 4 °C for 28 days, emphasize the zoonotic potential of poultry meat as a causative agent of human toxocarosis.

1. Introduction

The common roundworm of cats, *Toxocara cati* is a zoonotic helminth which is increasingly recognized as an etiological agent of human toxocarosis (Shimokawa et al., 1982; Fisher, 2003; Akao and Ohta, 2007; Lee et al., 2010). In addition to direct infection from eggs deposited to the environment, cultural dietary preferences for raw or undercooked chicken dishes may be associated with risk because chicken may serve as a paratenic host (Okoshi and Usui, 1968; Taira et al., 2011). Thus, accumulating evidence links human toxocarosis to consumption of raw or undercooked chicken products (Mitsugi et al., 1988; Nishikata et al., 1991; Yoshikawa et al., 2010). An experimental study clearly demonstrated that *T. cati* larvae in the chicken meat were highly infective to recipient mice (Taira et al., 2011).

As refrigeration or freezing of chicken products are common means of preservation through the consumer chain, an assessment of risk associated to consumption of *T. cati* infected chicken meat should include controlled experimental studies on the survival of muscle larvae at low temperatures. Although a few studies have indicated the survival of larvae of *Toxocara canis*, the round worm of dogs, at low temperatures, comparable knowledge does not exist for *T. cati*. Sprent (1953) reported that motile larvae of *T. canis* were found after digestion of mice carcass which had been kept at −20 °C for 4 weeks, although the infectivity of the larvae was not evaluated. Taira et al. (2004) reported also on *T. canis* that 43% of larvae from pig tissues and 19% of larvae from chicken tissues, both tissues were preserved at 4 °C for 1 week, were established in recipient pigs. In the present study, therefore, the infectivity of *T. cati*

* Corresponding author. Tel.: +81 42 850 2483; fax: +81 42 850 2483.
E-mail address: saitohya@azabu-u.ac.jp (Y. Saitoh).

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muscle larvae of chicken after storage at 4 °C and −25 °C was investigated in a mouse bioassay.

2. Materials and methods

2.1. Parasite isolates

Eggs of *T. cati* obtained from a naturally infected local cat were subsequently propagated in a laboratory cat. Adult worms expelled from this cat had typical arrow-head-formed cervical alae of *T. cati*. Fecal eggs were isolated, embryonated and stored in 1% formalin at 25 °C for approx. 3 months (Taira et al., 2011). The eggs were washed twice with tap water prior to inoculation to remove the formalin.

2.2. Chickens: infection and necropsy

Ten parasite-naive *Boris brown* chickens of both sexes, aged 4 weeks on the day of inoculation, were group-housed in disinfected cages. Food and water were administered *ad libitum*. Each chicken was inoculated orally with 10,000 embryonated *T. cati* eggs in a single dose of 0.7 ml egg suspension. All chickens were euthanized and necropsied at 30 days post-infection.

The pectoral muscles (white meat) and hindlimb muscles (red meat) of chickens were removed, and minced into pieces of approx. 5 mm^3 with a commercial food processor.

2.3. Preservation of the chicken muscle tissue

The entire portion of thoroughly mixed minced muscle tissue was divided into 18 portions of 50 g in plastic bags. All portions of minced muscle tissues in the plastic bags were flattened to about 2 cm in thickness. Three control bags were not stored and the muscle larval burden was assessed at the day of necropsy by HCl-pepsin digestion (see below). Five sets of three bags were stored respectively at 4 °C for 14 days, at 4 °C for 28 days, at −25 °C for 12 h, at −25 °C for 24 h, and at −25 °C for 48 h. The bags stored at −25 °C for 12 h were followed by 4 °C storage for another 12 h to digest the meats at the same time with that stored at −25 °C for 24 h. After storage, muscle larvae were immediately released by digestion as described below.

2.4. Mice: infection and necropsy

Thirty six 5 wk old male ICR mice (closed colony) were used in the study: Six groups of six mice were inoculated *per os* by stomach tubation with around 50 larvae per mouse (in approx. 0.5 ml saline). Inoculation was conducted within 1 h after the release of the muscle larvae. Fifteen days after inoculation, mice were euthanized. Whole carcass of a mouse, excluding the skin, tip of limbs, tail, muzzle, stomach, and intestines, were minced into pieces of approx. 5 mm^3 with a commercial food processor, and digested for larval recovery. The food processor was disinfected and washed with boiling water in the process for each carcass.

All animals used in this study were treated in accordance with the guidelines for animal experimentation of Azabu University with the reference number 100304-5 and the relevant ethical guidelines of the Japanese Ministry of Education, Culture, Sports, Science and Technology.

2.5. Digestion and larval counts

The muscle digestion was conducted after Taira et al. (2011): Chicken muscle tissue or carcass of mouse was digested in an HCl-pepsin solution for 2 h. The ratio between tissue (g) and fluid (ml) was approximately 1:10. The digestion solution was sedimented for 1 h and washed in 40 °C saline three times. Immediately after the final sedimentation, the recovered larvae were counted under a stereoscopic microscope, and their motility was roughly estimated.

3. Results

No clinical signs of the infection or changes in behavior were observed in the chickens and mice during the experiments. Macroscopic changes in muscles of chickens were not observed at necropsy.

The larval recovery from chicken meat and mice is presented in Table 1. Almost all larvae recovered from the chicken muscle tissue at necropsy (controls) were motile, and half (47.9%) of these larvae were recovered from the inoculated mice. Although, the muscle tissue did not show clear visible signs of degradation after storage at 4 °C for 14 days, only half of the released larvae were estimated as motile, and 24.1% of the inoculated larvae established in mice. After further storage at 4 °C for a total of 28 days, the tissue showed clear signs of degradation (appearance and smell), only a few of the released larvae were motile and overall only 3.3% of the inoculated larvae could be recovered from mice.

If frozen at −25 °C for 12 h, only a few of the larvae released from the chicken muscle tissue were motile, but none established in mice. When the storage period at −25 °C was extended to 24 or 48 h, the released larvae were neither motile nor infective to mice.

4. Discussion

The present study provides the first data on the infectivity of *T. cati* larvae in poultry after refrigeration or deep freezing of the meat. The bioassay in mice illustrates that *T. cati* larvae may retain high infectivity in chicken meat stored at 4 °C up to two weeks. An extended storage period significantly decreases larval infectivity. The decrease of larval infectivity may relate to decay process of the chicken meat, although the meats stored for 14 days did not show clear visible degradation. For human consumption, storage of fresh poultry would rarely exceed two weeks, and the present storage at 4 °C for 28 days may primarily be of academic interest. Thus, if stored in a common domestic refrigerator (4–5 °C), chicken meat infected with *T. cati* larvae may pose a food safety risk.

Contrary, deep freezing appears to be an effective measure to inactivate *T. cati* larvae in chicken meat, as none of the larvae recovered after freezing at −25 °C were able to establish in mice. The observation of a few motile