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Sex-Differentiating Criteria for *Trichinella spiralis* Muscle Larvae in Tissue Sections

YUZO TAKAHASHI, JUNKO FURUKI, YOSHIRO YOSHIKAWA, SHOUJI YAMADA, AND TSUNEJI ARAKI

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Abstract

New criteria for the differentiation of males from females of *Trichinella spiralis* muscle larvae were described in the present paper based on our histochemical observations of PAS- and AZAN-stained tissue sections. In histochemical staining profiles, a given muscle larva with the characteristic morphology of a female (short hindgut and uneven-sized germ cells) presented minute round spots that were PAS-positive or faintly blue-stained with AZAN in the genital primordium. In contrast, these minute round spots were absent in the genital primordium of a larva with the characteristic morphology of a male, that is, long hindgut and uniformed germ cells. These observations led us to conclude that a larva with such spots in the genital primordium is female and a larvae lacking the spots is male. The ability of PAS-and AZAN-staining to reveal the presence or absence of such spots makes these stains valuable techniques for sex differentiation of *T. spiralis muscle* larvae.

Key words: *Trichinella spiralis* muscle larvae PAS and AZAN stain sex

Introduction

Trichinella spiralis muscle larvae rapidly develop their primary sex characteristics and, therefore, the sex of a muscle larva can be determined as early as 10 days after intramuscular invasion (Kozek, 1975). Besides the genital organ, the number of stichocytes can be used as a mean of distinguishing males from females (Vittela, 1966).

In this study, longitudinal sections of muscle larvae of *T. spiralis* were performed according to a technique established in our laboratory (Takahashi, 198). The use of Acrytron E, a hydrophilic embedding material, not only allowed hematoxylin-eosin (HE) staining, but also a variety of high-resolution histochemical staining. During examination of a histochemical profile of a *T. spiralis* muscle larva, PAS- and AZAN-staining incidentally revealed the existence of two groups in the population of *T. spiralis* larvae under investigation. One group had minute round spots which were PAS-positive and stained very light blue by AZAN in the genital primordium, while the other group was devoid of such spots. We speculated that this difference in staining pattern may reflect a difference in cytochemical characteristics between male and female larvae. To ascertain this hypothesis, possible correlations were examined between the histochemical staining profile and the length of the hindgut by which sex of *T. spiralis* larvae can be determined (Kozek, 1975; Despommier, 1983).

Department of Parasitology, Nara Medical University, Kashihara, Nara 634, Japan

高橋優三　古木純子　吉川義朗　山田祥次　荒木恒治
(奈良県立医科大学寄生虫学教室)

Materials and Methods

T. spiralis larvae (Polish strain kindly supplied by Prof. T. Yamaguchi, Hirosaki University, School of Medicine) were maintained in ICR mice. Muscle larvae of more than 1 month post-infection were recovered by pepsin-HCl digestion (Despommier, 1974). The isolated larvae were squashed and fixed with half-strength Karnovsky solution (Karnovsky, 1965), dehy-

drated, embedded in Acrytron E (Mitsubishi Rayon Co., Ltd., Tokyo) according to our previously described method (Takahashi, 198). Longitudinal sections were then made and stained with either PAS or AZAN following standard techniques. The length of the hindgut was estimated by comparing it with the diameter of the parasite at the intestine-rectal junction.

Results

A non-contractile portion of the body wall muscle was PAS-positive and stained in an intermittent linear fashion, and the cord and the epithelial cells of the gastrointestinal tract stained in a spotty pattern (MG and HG in Figs. 1 and 2). The amorphous material within the ampulla portion of the midgut was weak-positive for PAS (data not shown).

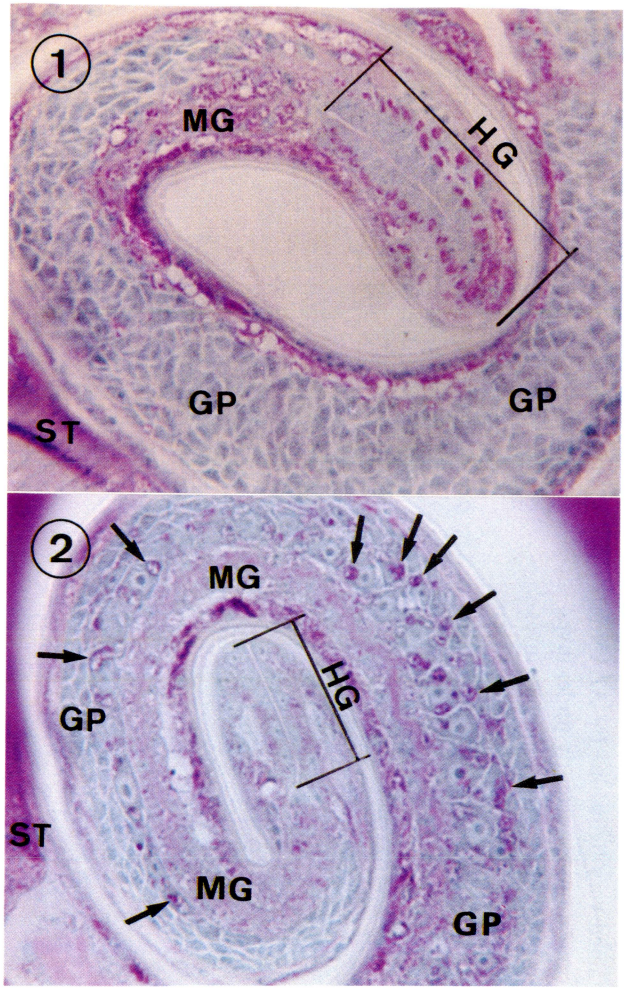
A heterogeneous staining pattern was observed as to the genital primordium. Even adjacent worms in a single section showed different staining pattern by PAS-staining. Some of the larvae presented PAS-positive spots in the genital primordium (Fig. 2), whereas other larvae did not (Fig. 1). The hindgut length of the larvae from each group was examined in a longitudinal section of the hindgut allowing comparison of the length of the hindgut with the diameter of a given larva at the intestine-rectal junction. In the group of larvae with short hindgut with a length approximately equal to the diameter of the larva at the intestine-rectal junction, the genital primordium had PAS-positive spots (Fig. 2). On the contrary, PAS-positive substances were not observed in the group of larvae with long hindgut, with a length between 1.5 to 2 times the diameter of the larvae at the junction (Fig. 1). Additional findings in the short-hindgut group were that these larvae presented genital primordial cells of uneven size, the smallest cells being located in the dorsal portion, while the largest and round cells were present in the ventral portion of the genital primordium (Fig. 2).

The AZAN-staining gave an impressive staining pattern. When AZAN-staining was carried out on the Acrytron-embedded larvae, the cuticle stained red, and the stichocyte granules stained blue or red (Figs. 3 and 4). All of the genital primordial cells examined stained yellow or orange yellow. Of particular interest was that the larvae with short hindgut had minute round spots that stained very light blue in the cytoplasm of the genital primordial cells and these cells tended to be of uneven size (Fig. 4). This very light blue staining (sometimes the staining was barely visible) was distributed in a similar fashion as described for PAS-positive substances, and could be distinguished from the blue staining of the stichocytes (ST in Figs. 3 and 4). The larvae with the long hindgut lacked these minute round spots in their genital primordial cells and these cells tended to be of even size (Fig. 3). Thus equivalent results were again obtained by AZAN-staining. The results obtained by PAS- and AZAN-staining were confirmed in more than 10 larvae from each group.

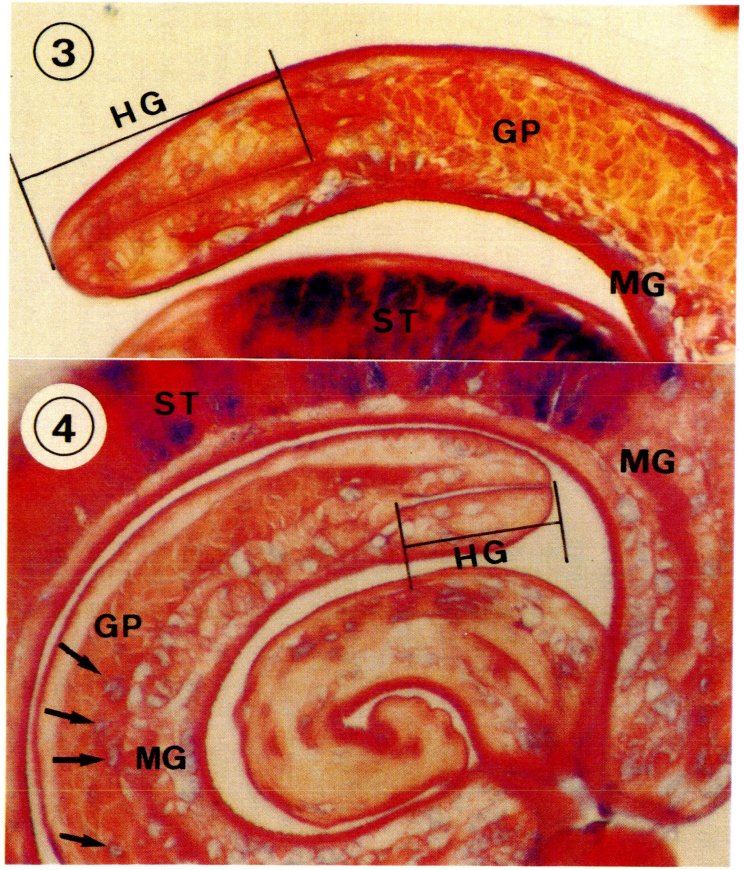
Discussion

Our recently described method of "squashing and fixation" allowed us to make longitudinal sections of *T. spiralis* larvae (Takahashi, 198). The length of the hindgut was estimated by comparing the length of the hindgut with the diameter of the worms in the longitudinal tissue section which is cut through the center of the worm. After this estimation was made, a definite correlation was appreciated between the length of the hindgut and the histochemical staining profile of the genital primordium. Larvae with short hindgut (equal to their diameter) had PAS-positive spots in the genital primordium. By contrast, larva with long hindgut (1.5 to 2 times their diameter) did not present PAS-positive spots.

According to Kozek's thesis (Kozek, 1975), the length of the female hindgut is approximately equal to the diameter of the larva at the intestine-rectal junction, and the length



Figs. 1 and 2. Semithin sections of Acrytron-embedded muscle larvae were stained with PAS. The larvae with long hindgut exhibited PAS-positive substances (arrows) in the genital primordium (GP) (Fig. 2), while the larvae with short hindgut failed to exhibit such substances (Fig. 1).



Figs. 3 and 4. Semithin sections were stained with AZAN. Arrows indicate minute spots in the genital primordium (Fig. 4).

Abbreviations: the hindgut (HG), the midgut (MG), the stichosome (ST), the genital primordium (GP).

of the male hindgut is 1.5 to 2 times its diameter. This striking difference between male and female larvae in turn raises the possibility that *T. spiralis* larvae can be sexed in tissue sections based on the following histochemical criteria, that is, a larva with PAS-positive spots in the genital primordium is female, and a larva lacking these spots is male.

This histochemical criteria seem to be further supported by another line of evidence that a larva with the PAS-positive spots had genital primordial cells of uneven size, which is one of the morphological characteristics of female larvae (Gould, 1945; Kozek, 1975; Despommier, 1983). On the other hand, a larva lacking PAS-positive spots had uniformed genital primordial cells which is the characteristics of male larvae.

Our AZAN-staining experiments also revealed the same pattern observed with PAS-staining. Consequently, larvae with short hindgut and uneven-sized germ cells (characteristics of females) showed minute round spots which stained very light blue, and larva with long hindgut and uniform-sized germ cells (characteristics of males) were avoid of such minute round spots.

The exact nature of PAS-positive spots and those that stained very light blue by AZAN in the genital primordium is currently under investigation in our laboratory. A preliminary investigation revealed that these spots are glycogen aggregate present in the cytoplasm of the genital primordial cells.

In conclusion, our observations clearly establish steady correlation between the presence of such spots in the genital primordial cells and female larvae, and between absence of such spots and male larvae. Since PAS- and AZAN-staining reveal the presence or absence of minute round spots with ease and reliability even in randomely selected sections, PAS- and AZAN-staining profiles may now be included in the current list of characteristics for distinguishing males from females.

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