# Detection of white mice intestinal immune response against the external surface of *Hymenolepis nana* by scanning electron microscope

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## Abstract

The aim of the study was involve the scanning electron microscope to detect any damage to the surface of the *Hymenolepis nana* worm at different ages due to host intestinal immune response. Fifteen male albino Balb/c mice were used in this experiment. The eggs suspension obtained from gravid proglottid was given to mice by administration tube. The infected mice were killed at different intervals. The intestines of mice were opened and the worms allowed to release. These worms were examined by electron microscope. The results as observed; the surface of the scolex has a smooth structure in both 30 and 63 days old worm. The neck surface was smooth at 10 days and at the 30 and 63 days old worms was pitted. There were puff-like cells attached to the surface; these were lymphocytes, and may be eosinophil. Similar damage was seen on the tegument of posterior part of the worm. We can conclude that there is a progressive destruction of tegument as worm ages, and this is believed to be a consequence of immune response.

**Key words: Scanning electron microscope, immune damage, tegument, mice, *H. nana*.**

# الكشف عن مظاهر الاستجابة المناعية المعوية للفئران المهق تجاه سطح الدودة الشريطية المحرشفة القزمة بواسطة المجهر الاليكتروني التفراسي

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## الخلاصة

هدفت الدراسة الى استخدام المجهر الالكتروني التفراسي للكشف عن الضرر الحاصل لسطح الدودة الشريطية المحرشفة القزمة المخمجة للفأر الامهق لمختلف الاعمار نتيجة الاستجابة المناعية المعوية ، استخدمت في الدراسة 15 من ذكور الفأر الامهق. اعطي للفأر عن طريق انبوب الاعطاء معلق البيوض المأخوذ من القطع الحبلى للدودة ، وقتلت الفئران المخمجة على فترات زمنية مختلفة ، وفتحت امعاء الفئران وجرى تحرير الديدان وفحصها باستخدام المجهر الالكتروني. لوحظ من النتائج إن رويس الدودة كان أملسا في عمر 30 و63 يوما ، أما عنق الدودة فقد كان أملسا في عمر 10 أيام في حين كان منقرا في عمر 30 و63 يوما. لقد شوهدت خلايا نفيشة قد تماست مع سطح العنق والتي هي خلايا لمفاوية إضافة إلى خلايا أخرى قد تكون من الحمضات كما إن ضررا قد لحق بالجزء الخلفي من لحافة الدودة ، أما الشدفة الطرفية الخلفية في عمر10 أيام فقد كانت ملساء ومنقرة ، في حين كانت في عمر 30 و 63 يوما ( والتي من الممكن ان تنفصل عن جسم الدودة ) فأن سطحها يكون منقرا بحاله شبيهه بما موجود في العنق ووجود خلايا يظن أنها من الحمضات والتي تشاهد غالبا بأعداد كبيره.

**الكلمات المفتاحية: المجهر الالكتروني التفراسي ، الضرر المناعي ، السقيفي ، الفأر الامهق ، الدودة المحرشفة القزمة .**

## Introduction

The tapeworm *H. nana* is found in the tropics and subtropics areas, it commonly occurs in children and in institutionalized people living in close quarters (1). The expulsion of *H. nana* from the intestine of the mouse is due to an immune response. Therefore it seemed of interest, to use the electron microscope to see if there is any damage similar to that reported by (2) in *Hymenolepis diminuta* from rats, and if so, to determine the timing of the appearance of this damage. The structure of the cestode

tegument is particular interest to helmintho-logist, as these worms lack a mouth and digestive system and, therefore, all nutrients must pass across the body wall. The body covering is thus a metabolically active surface which plays an important role to physiology of these organisms (3, 4). Most of the previous work on the ultra-structure of the cestode tegument has been carried out on cyclophyllidean tapeworms; including *H. diminuta* and *H. nana.* (5) was the first to investigate details about the embryonic syncytial epithelium of *H.diminuta* (6), while (7) described the surface structures of *H. diminuta*. The tegument surface of *H. nana* and *H. diminuta* is fine structure and is densely covered with microtriches which are of the same shape and were never seen to be branched (8). The aim of the study is to involve scanning microscope to detect any damage to the surface of the worm due to host immune response.

## Materials and methods

Fifteen male albino Balb / c mice aged 8-10 weeks and weighing 20-25 gm (from the animal house of Al-Nahrain University) was utilized.

**Parasite:** The parasite (*H. nana*) was obtained from the College of Veterinary Medicine / Baghdad University.

**Infection of mice with *H. nana* eggs by stomach tube.**

Gravid proglottid obtain from an already infected mouse were transferred to a pestle containing normal saline, and ground gently with a mortar. The egg suspension was allowed to stand for a few hours and then it was withdrawn carefully by pipette, preventing the sediment from mixing with the supernatant. The egg suspension was then shaken vigorously and three 0.1 ml aliquot were taken by graduated syringe; each 0.1 ml was placed on a clean slide and the eggs were counted in each aliquot. The mean was taken and dilutions made to any required concentration of eggs (9). The solution was then given to the mice by administration tube. The infected mice were killed at different intervals. The intestine was opened in a Petri dish filled with normal saline the worms were released and allowed to relax.

**Scanning electron microscope preparation**

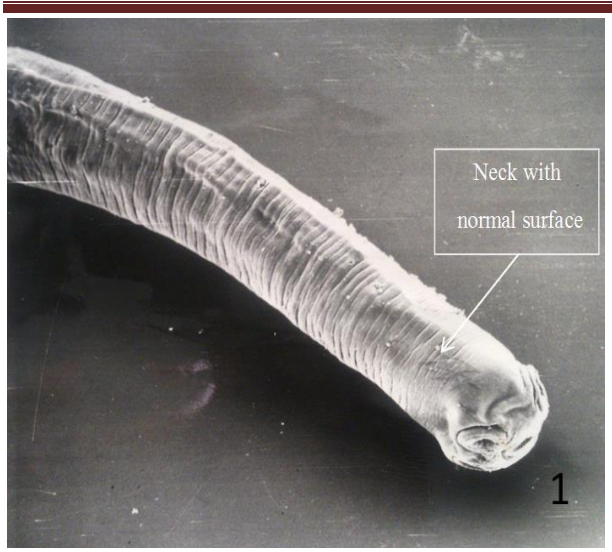
The worms were rapidly dehydrated in descending series of ethanol solution, transferred through a series of intermediate fluid (3:1, 2:1, 1:1) of 100 % acetone. Specimens were transferred to liquid $CO_2$ in a polaron CPD-1 critical point dryer until the acetone was removed. The dried specimens were positioned on metal specimens tubs, rotary coated with gold palladium and then coated in ( polaron sputter coater ) with gold ( 200 $A^O$ or less ) and examined with Cambridge-54 scanning electron microscope / Bristol University / England.

## Results

As observed under the scanning electron microscope, the surface of the scolex has smooth structure in both 30 and 63 day old worms (Fig. 1, 2). In 10 day old worms the neck is equally smooth (Fig. 3), but in 30 and 63 day worms the neck surface is pitted (Fig. 4). There is puff like cells attached to the surface; these are lymphocytes and may will be eosinophil (Fig. 5). Similar damage is seen on tegument of the posterior part of the body. The original terminal proglottid in a 10 day old worm (Fig. 6) is smooth and pitted, but in 30 and 63 day worms the surface of posterior proglottid (the original terminal proglottid has by now been shed) was pitted in the same way as observed in the neck (Fig. 7) and presumed eosinophil can be seen (Fig. 8), often in large numbers.

## Discussion

Structural changes to parasites within the gut which appear to be a result of host immunity have been described. The most important paper is that of (2) who showed that the immunological damage can occur in *H. diminuta*. This is includes increasing amounts of lipids, abnormal mitochondria, and dark areas on the surface of worm which had been incubated in Hanks' saline. The results presented here confirm some of (2)



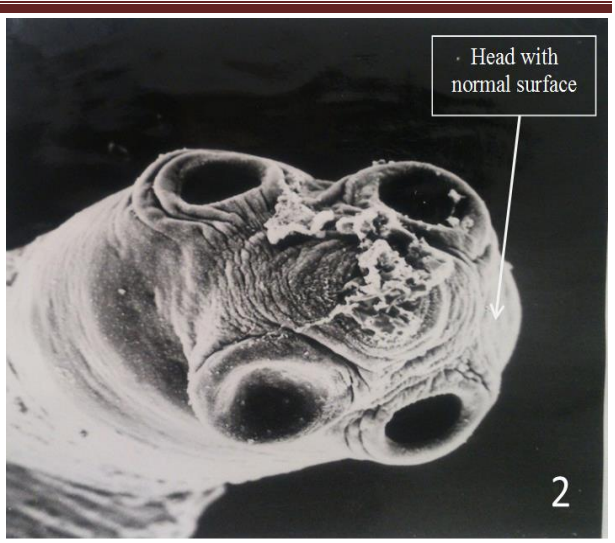
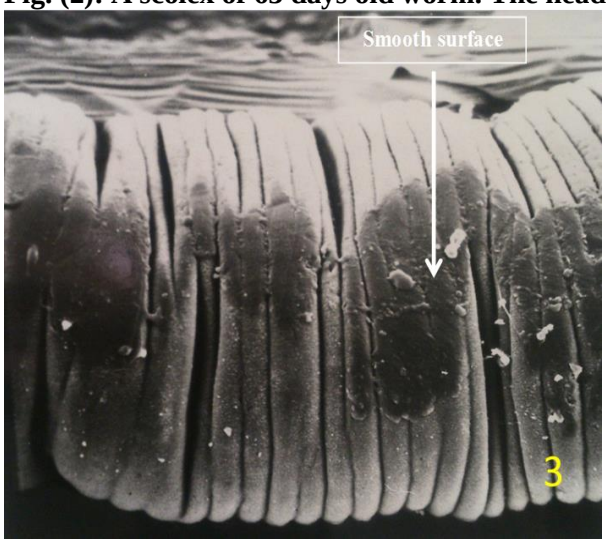




**Fig. (1): A scolex of 30 days old worm which shows no damage. X550.**
**Fig. (2): A scolex of 63 days old worm. The head appears normal. X1000.**



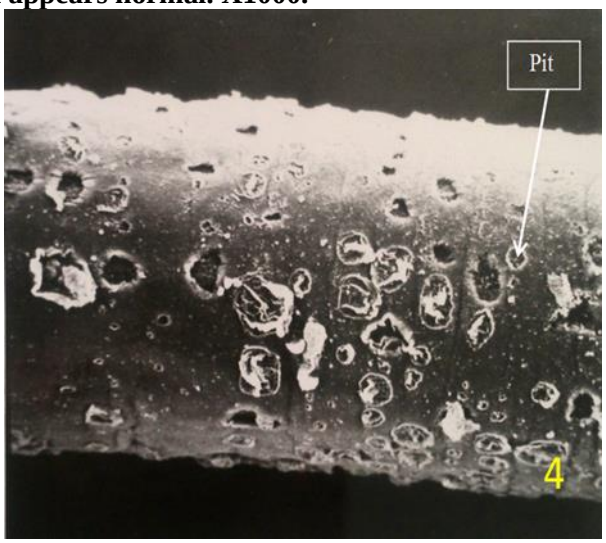
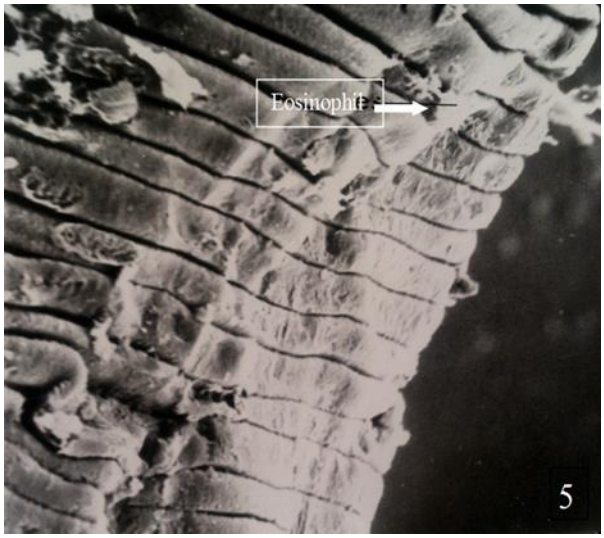




**Fig. (3): Neck of 10 days old worm. The normal smooth surface. X1000.**
**Fig. (4): Neck of 63 days old worm. The surface appears uneven and the number of pits has increased. X1100.**



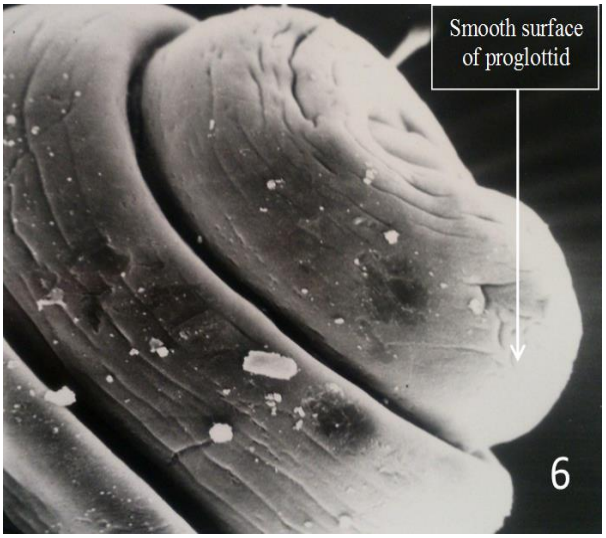




**Fig. (5): Neck of 30 days old worm. The surface appears damaged with disc- shaped pits, and there is an eosinophil, which appears as a puff shape provided with branched projections. X2000.**
**Fig. (6): Terminal proglottid of 10 days old worm. The surface appears smooth and even. X650.**


**Fig. (7): Posterior proglottis of 30 days old worm. Note the surface damage and the presence of pits. X500.**

**Fig. (8): Part of posterior proglottids of 63 day old worm. Note that pits are larger in size, and the large number of attached eosinophil. X2450.**

finding, but they also add much new information. First of all, it is clear that tugumental cells become damaged. No attempt was made to examine the surface from the standpoint of immunochemistry, so it not known whether *H. nana* acquires an immunoglobulin coat. (2) reported dark areas on the surface of immune - exposed *H. diminut*a. No such areas were seen in the present study, but perhaps they are equivalent to the pits seen in fig. 5, 6, 7 and 8 when they were first observed it is not known how these pits might have been caused, but they seemed to represent some kind of damages to the surface of the worm. The recognition of lymphocyte like cells, which are probably eosinophil, on the surface of older worms, when the scanning photographs were examined immediately suggested an explanation. The eosinophils are probably attacking the surface, as they are known to do in Schistosomes (10). lt is not known why there is no damage to the scolex in older worms. This may be a consequence of the fact that the scolex is buried in the spaces between villi, and so they may not be exposed to the complete range of cellular, enzymatic, inflammatory and humoral components of the intestinal immune response.

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