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SCANNING ELECTRON MICROSCOPIC EXAMINATION OF THE TEGUMENTAL SURFACE OF TWO PARAMPHISTOMID SPECIES (TREMATODA: PARAMPHISTOMIDAE)

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İki Paramphistomun Türünün Tegumental Yüzeylerinin Skaning Elektron Mikroskopik Bakısı.

Özet: *Bu araştırmada taksonomik geçerlilikleri konusunda halen tartışmaların devam ettiği iki Paramphistomun türünün (Paramphistomum cervi Zeder*, 1790 *ve P. leydeni Näsmark*, 1937) *tegümental yüzeylerini kontrol etmek ve genital delik yapılarındaki histomorfolojik özellikleri karşılaştırmak amaçlanmıştır.*

Muayene edilen 24 *parazitten* 6 *sının yüzey yapısında farklılıklar gözlenmiştir. Ancak, bu parazitlerin genital delik yapılarında önemli bir fark bulunamamıştır. Bu sonuçlar, P. leydeni'yi P. cervi'den ayırımda kullanılan kriterlerin süreklilik ve güvenilirliklerine şüphe düşürmüştür.*

Summary: *The present investigation was carried out to investigate the tegumental surfaces and to compare the histomorphological pecularities of genital openings of two Paramphistomid species (Paramphistomum cervi Zeder*, 1790 *and P. leydeni Näsmark*, 1937) *which their taxonomical validity are still under consideration.*

The individual variations could be observed on the surface topography of 6 *parasites out of* 24 *examined. However, no significant difference were found in the genital opening structures. These results cast doubts on the consistency and reliability of criteria used in distinguishing P. leydeni from P. cervi.*

Introduction

The taxonomy of the family Paramphistomidae was based on the histomorphological pecularities of certain muscular organs (parti-

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cularly pharynx, genital opening and acetabulum) as seen in median sagittal sections. Näsmark (3) had described Paramphistomum leydeni as a new species, and separated it from P. cervi by having a well developed genital opening (Leydeni type g.o., Sensu Näsmark). In later years, Sey (5), after examining a large collection of Amphistomes from the different european sources and Näsmark's (3) original preparations, has refused the validity of P. leydeni and proposed that this species should be regarded as a synonym of P. cervi. This synonymisation had already been accepted by Odening et al. (4).

In recent years, scanning electron microscopy (SEM) has thrown a new light upon the taxonomical studies. In the case of Paramphistomid species which are sometimes morphologically identical, the usage of SEM took a special place in distinguishing these species. Eduardo (1,2), for the first time, has announced that many closely related species could be separated from each other by their surface structures. On this basis, he has separated P. leydeni from P. cervi by the presence of dome to conical shaped tegumental papillae which were concentrated anteriorly around the oral opening and ventrally around the genital pore. P. cervi has been described as completely without papillae. Eduardo (2) has also confirmed this separation histomorphologically by describing new traits in the genital opening of P. leydeni. In his descriptions, Leydeni type of genital opening (P. leydeni) has been characterized by having thick and large genital papillae and strongly developed radial fibres. However, Gracile type of genital opening (P. cervi) was characterized by being weakly developed and presence of only few radial musculature.

Recently, scanning electron microscopical examinations on the surface of P. cervi revealed that this species has also carried tegumental papillae (6). However, the type of tegumental papillae of P. cervi (short and stumpy papillae, sitting on a tegumental elevation. Sensu Sey) were not identical with those of P. leydeni. Thus, the taxonomical situation and identification of these species has become confusing.

The following possibilities were submitted by Sey (6) on this subject.

1- If any special importance is attributed to the tegumental papillae, in that case P. cervi and P. leydeni are valid and has to be considered two different species.

2– If tegumental papillae have considerable indivudial variations, in that case P. leydeni is a synonym of P. cervi.

While the above authors were indicating the taxonomic value of tegumental papillae, Tandon and Maitra (7) emphasized their physiological (host-parasite interface relationship) importance.

With the present study we examined the pattern and consistency of tegumental papillae of both species and compared their genital opening structures.

Material and Methods

The majority of the Paramphistomum species were obtained from the rumens of both sheep and cattle slaughtered in Ankara abattoir recently with the exception of one group of parasites which had been obtained from sheep slaughtered around 10 years ago.

The samples were directly fixed in 70 % alcohol except the old group which had been fixed and stored in 10 % formaline.

Five Paramphistomid specimens were sectioned in median sagittal plan from each group, carrying 8–12 μ thickness, and stained with haematoxyline and eozin in usual microtechnical way.

A total of 8 group of parasites (6 from sheep and 2 from cattle) comprising. P. cervi and/or P. leydeni were separated for this investigation. From these groups, 24 parasites (3 from each group) were dehydrated through an ascending series of alcohol, transferred to amylacetate-alcohol mixtures, gradually up to absolute amylacetate. Then they were critical point dried in liquid CO_2 and coated with platinpalladium in a sputtering unit (Eiko IB-3 Ion coater). Specimens were examined in JEM 100-B-ASİD-1 scanning electron microscope at 20 Kv.

Moreover, in order to examine the genital opening structure and surface topography of the same specimen, three parasites showing different surface topography were separated under stereo microscope (x 80). Each of these parasites was sectioned with microtome until the features seem to be sufficient for histomorphological diagnosis. Then the remained parts of the same parasites were differentiated in xylol, followed the above mentioned method, and then examined in SEM. This method gave us the oppurtunity to study each specimen histomorphologically and with scanning electron microscopically.

Results

Sixteen out of 24 parasites examined in SEM showed from dome to conical shaped tegumental papillae extending to the genital opening-level (Fig. 1). Only two parasites from the old group were completely without papillae (Fig. 2). However, the rest 6 parasites showed some variations on their surface topography as listed below.

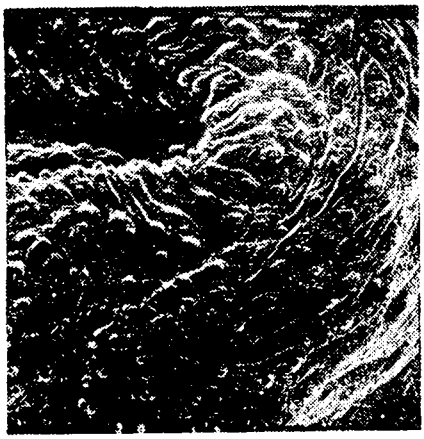

Fig. 1. Dome to conical shaped tegumental papillae on the body surface.

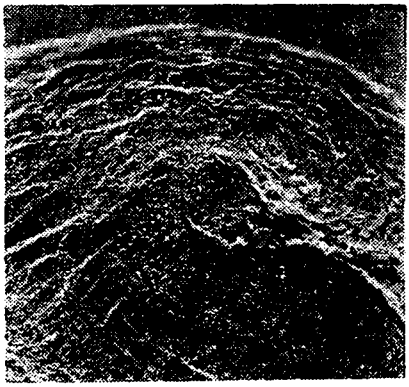

Fig. 2. Note the absence of papillae on the surface of the body.

1– Three parasites showed hardly emevated and randomly arranged few papillae on the anterior end around the oral openings (Fig. 3).

2– Two parasites were carrying papillae located anteriorly around one fourth of the body lenght. These papillae never extended ventrally to the genital opening-level (Fig. 4).

3– One parasite showed only a few papillae on anterior half of the body (Fig. 5).

Among the parasites examined, the rate of individual variations of tegumental papillae seems to be worth attention.

In the histomorphological examinations of the genital openings of these species it was observed that there was no a high correlation between the thicknesses of genital papillae and development rate of radial dibres.

Fig. 3. Hardly elevated and randomly arranged few papilae on the anterior end around the oral opening.

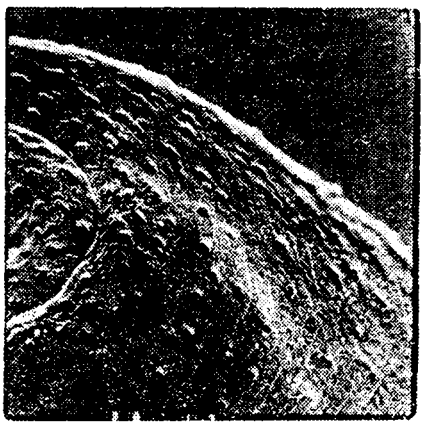

Fig. 4. Papillae located anteriorly around one fourth of the body lenght.

As seen in Fig 6, in some genital openings with thick genital papapillae radial fibres were weakly developed or completely absent. On the other hand, sometimes it was difficult to determine whether genital papillae were thick or thin in a genital opening.

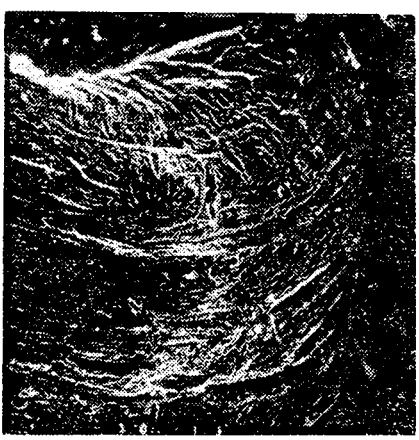

Fig. 5. Note the presence of only a few papillae on anterior half of the body.

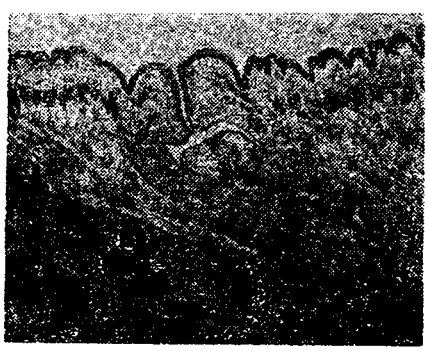

Fig. 6. Gracile type of genital opening with thick genital papillae.

In addition, not a good correlation could be observed between the presence of tegumental papillae on the surface of the body and muscular structure of genital opening of the same specimen. As seen

in Figs. 7a and 7b, although presence of tegumental papillae on the surface of the body, there was only a few radial fibres in the genital opening of the same specimen.

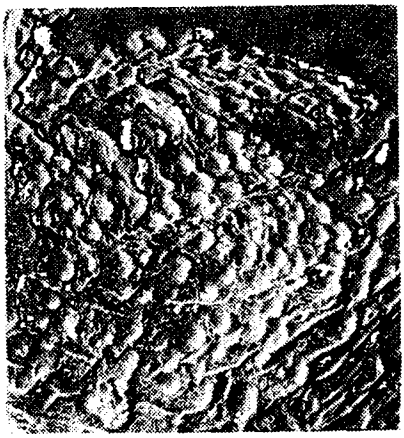

Fig. 7a. Surface topography of a specimen with tegumental papillae.

Fig. 7b. Genital opening structure of the specimen illustrated in Fig 7a. Note the presence of only a few radial fibres.

Discussion and Conclusion

Our observations on the consistency of tegumental papillae somewhat deviated from Eduardo's (1, 2) findings. The individual variability on the surface topography of these species could be observed at a rate of that should not be overlooked.

On the other hand, no parasites resembling to Sey's (6) P. cervi could be observed in this study.

It is of interest to point out that only the parasites from old group were entirely without papillae. A definite explanation for the waste of these papillae is far from being certain. It seems that the prefixative treatment, storage time and condition for these parasites could be the reason.

Two out of 6 parasites showing variations on their surface topography may give the impression that these parasites are P. ichikawai (Fig. 4). However, the presence of long papillae through the inner surface of pharynx obviously reveals the fact that they are not P. ichikawai (Fig. 8).

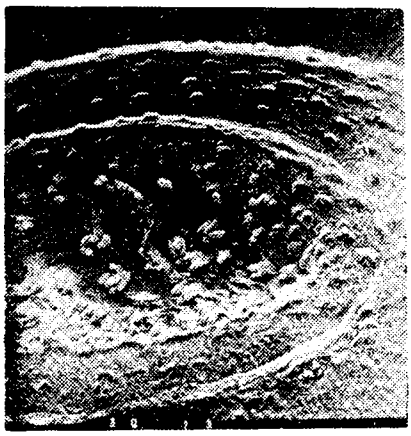

Fig. 8. Long papillae through the inner surface of pharynx of the specimen illustrated in Fig. 4.

In histomorphological examinations, contrary to Eduardo's description on Leydeni type g.o., weakly developed radial fibres were observed. On the other hand, absence of a numerical value for the thickneses of genital papillae made sometimes difficult to determine whether they were thick or thin.

These results have cast doubts on the criteria used to separate these species from each other.

Comprehensive studies are necessary to elucidate the incidence of variations on the surface topography of these species and to detect the precise intermediate host (s) of these parasites to consider them as different species.

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