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CYTOMORPHOLOGICAL MODIFICATIONS OF INTESTINAL EPITHELIUM OF BEE (*APIS MELLIFERA*) PARASITED BY *NOSEMA* SPP.

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Abstract

The work aims to investigate the histological changes produced by the parasite Nosema spp. in bee species Apis mellifera, using as a liquid fixative Dubosq-Brasil and Masson modified coloration.

Key words: *histological techniques, intestinal epithelium, bees, Nosema spp..*

INTRODUCTION

Apis mellifera is a pollinating insect constantly exposed of different pathogens especially parasites (Webster et al., 2008, Bailey and Ball, 1991). Among these parasites are microsporidians from *Nosema* genre. This represents a numerous group of endoparasites with intracellular localisation, who presents a unicellular structure and have the capacity to make spores (Forsgren and Fries 2010). The election place of all species from *Nosema* genre is represented by the cytoplasm of epithelial cells of digestive mucous tube of adult bee (Webster et al., 2004).

MATERIALS AND METHODS

Initially, we aimed to realise the histopathological examination on living bees samples with scope to observe tissue modifications as consequence of viral pathogens agents possible transferred by *Nosema spp,* by kind of Morison corps.

In order to determinate existent modifications at intestinal epithelium level, consecutive hemolymph depletion produced by parasitism with *Nosema*, it was created a probe stamp with living bees gathered from study apiary, respective experimental apiary of Institute for Diagnosis and Animal Health (IDAH) and have been created two samples, sample I which consisted in bees with no clinical signs of any diseases, where parasitological, bacteriological and mycological examinations, didn′t evidenced the evolution of specifically disease and sample II, which consisted in bees from colonies with nosemosis .

The samples have been obtained at the end of active season, in October 2012. Every sample, for each Apiary consisted in 25 g living adult bees parasitized by *Nosema.* For sample creating there have been selected bees which has inserted 1-2 Nosema parasites between abdominal tergites, and one of them presented wings malformations or small abdomen.

All bees in the Group II were from honeybee colonies that had isolated brood cells presenting irreversible changes in physical characteristics, of the nymphs and the hatching of bees. Digestive system samples collected from bees belonging to consignment have been fixed in the effectiveness of the jumpers: formalin, Dubosq-Brasil, Carnoy and Lille and at the optimal time interval, i.e. at least 72 hours.

All permanent histological preparations were examined from optical microscope Zeiss, with magnification power of 150 X to X 1250, transmitted light with AxioVision system for capturing microscopic and computational processing them with IDAH: license 'Zeiss Axioplan 2 Image'.

Stage of dehydration and pre including the paraffin was done automatically by means of a Pathcentra device, and inclusion in paraffin wax anatomical parts was also automatically with the help of Kunz W – 4.

In order to obtain the microtomical sections has been used Finesse microtome, for laying them, thermostatically controlled bath Kunz set at 40 ° C and a hob with electronic adjustment.

Fixed anatomical pieces were processed and analyzed in the Laboratory of pathology and health of IDAH useful Insects.

The method used was represented by the specific histopathological techniques.

Dubosq– Brasil mixture, formalin in aqueous solutions of 10%, Carnoy-mixture and Lillie mixture were used for histological preparations obtaining.

Fixing of parts of the digestive system's Anatomy of honey bees (gut, the gut environment together with the insertion of Malpighi tubes and posterior intestine) has been carried out immediately after the harvesting a span of 15 minutes;

The ratio of the liquid Fixer and anatomical parts was at least 1/20 and 1/40.

RESULTS AND DISCUSSIONS

Histologically, viral inclusions are highlighted in the Morison epithelial cells of the small intestine, intracytoplasmic. They must be in the form of corpuscles, rounded 1-2 micrometers in diameter, opaque, more numerous in the area of insertion of Malpighi tubes. Also, the spores of *Nosema spp*, causing destruction of the intestinal epithelium, and do not turn entirely through Mason changed colour.

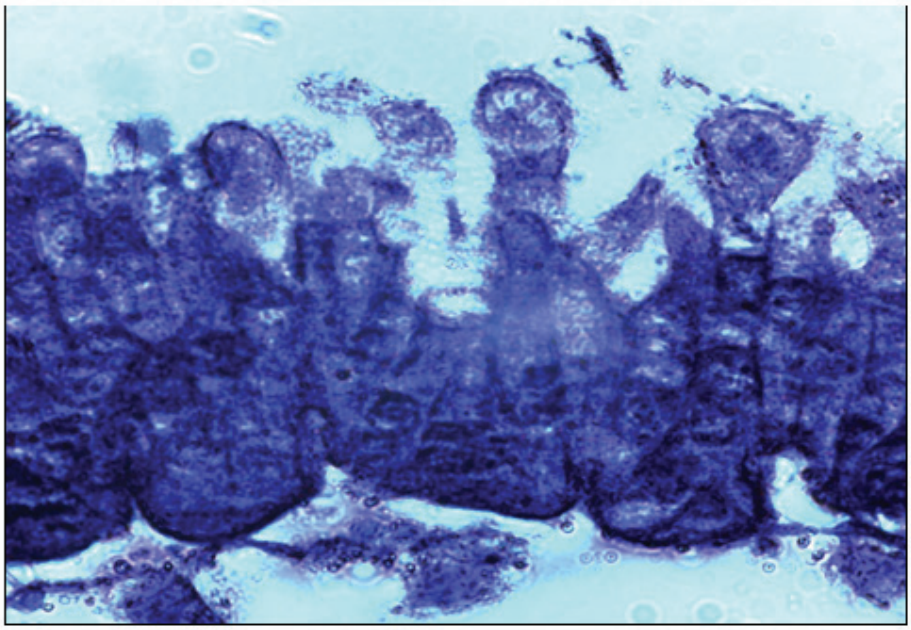

Figure 1. Intranuclear microvacuolisations and intracytoplasmic, with the apical epithelial cell lysis. Masson staining method, Carnoy fixation , X 1000. (original)

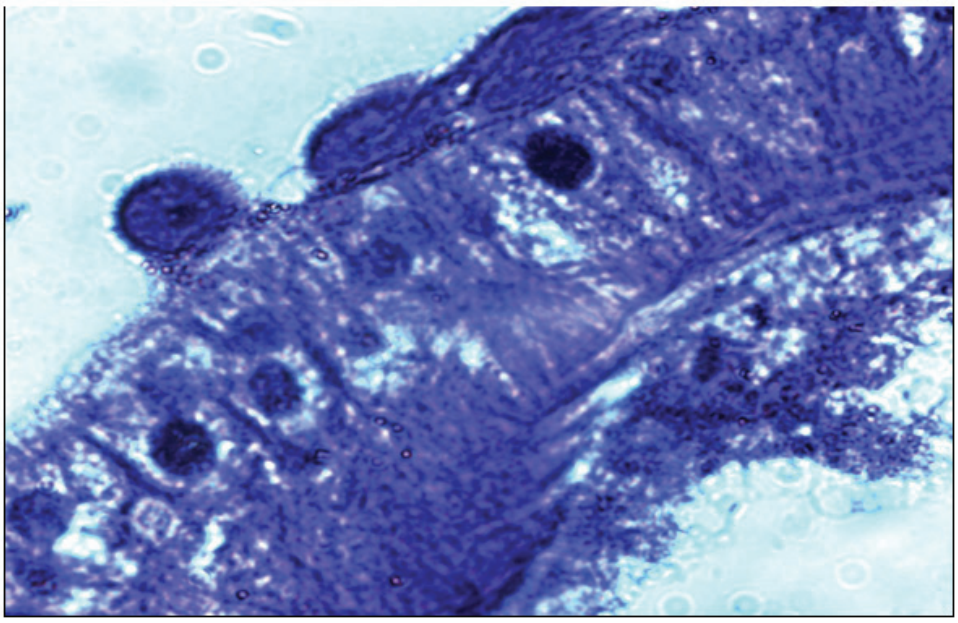

Figure 2. Granular and vacuolar alteration-confluante in the cytoplasm of epithelial cells. Thin bowel adult bee. Modified Masson staining method, in liquid Lilie fixation X l000 (original).

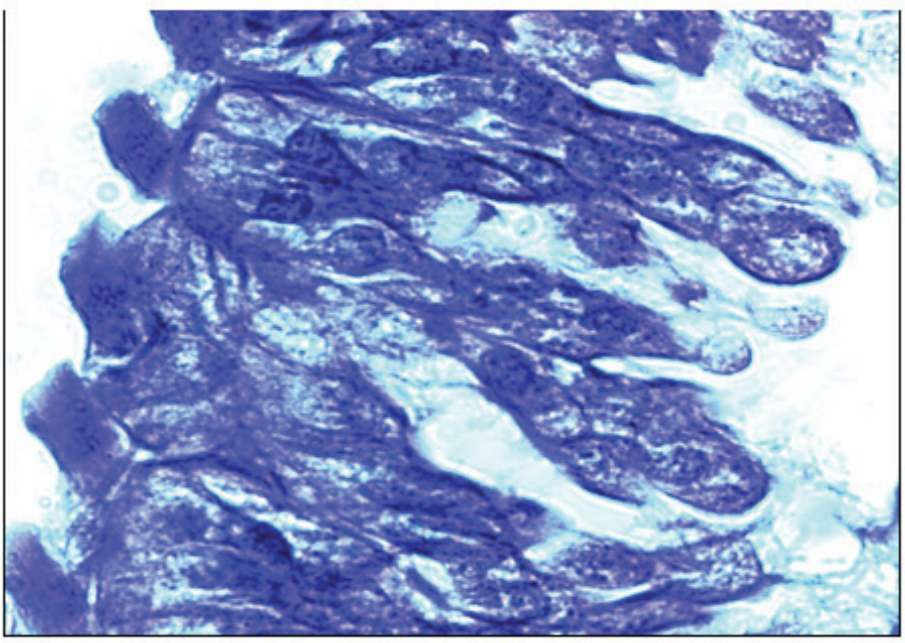

Figure 3. Medium intestine, the adult bee. Modified Masson staining method in liquid Dubosq-Brasil, x 1000, without filter NG (original)

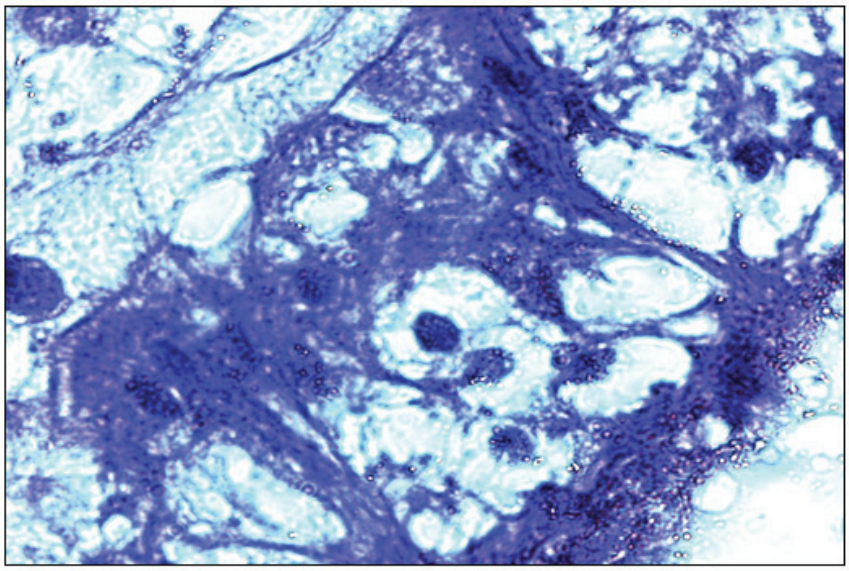

Figure 4. Vacuolisation and cytoplasmic aggregation, cariolisis, with dilaceration of the basal membrane. Infection with spores of Nosema spp., adult bee

Liquid fixative Dubosq-Brasil and modified Masson method had shown the structure and and cellularity of medium intestine and also the structure and characteristic of Malpighi tubes.

The histopathological examination done has allowed level of infection establishment with Nosema spp, which produced irreversible modifications of intestinal epithelium structure, as a result of digestive function alteration in bees and finally the bee′s death.

In the process of examining histological preparations obtained from samples of bees belonging to the lot on intestine and fastened with solutions, they noticed a lot of changes (microvacuolisations, lysis, epithelial cells, etc.) in the structure of small intestine epithelial cells and thin.

CONCLUSIONS

Fixing with the utmost efficiency and colouring Dubosq-Brasil Masson changed have highlighted in the control group, another type of histological changes that can be translated through a proliferative process observed only in the small intestine epithelium, the environment where intense metabolic reactions take place

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