Acta Veterinaria (Beograd), Vol. 50. No. 4, 253-262, 2000.

UDK 619:638.015-085

THERAPEUTIC EFFECTS OF FUMAGILLIN AND FIRST APPEARANCE OF *Nosema apis* SPORES FOLLOWING INFECTION

V. MLAĐAN*, D. TODOROVIC** and MIROSLAVA LOLIN**

*Institute of Veterinary Medicine of Serbia, Vojvode Toze 14, 11000 Belgrade, Yugoslavia **Faculty of Veterinary Medicine, University of Belgrade, Bulevar JNA 18, 11000 Belgrade, Yugoslavia

(Received 4. April 2000)

The therapeutic effect of Fumagillin after artificial infection of bees with Nosema apis spores was investigated. The appearance of spores and development stages of Nosema apis in the midgut after infection was detected pathohistologigally.

Three groups of bees were set up:

Group A was artificially infected with N.apis spores in sugar syrup;

Group B - On the 8th day following infection bees were administered sugar syrup with Fumagillin. The first application included 0.005 g Fumagillin DCH in 20 ml of syrup. Identical therapy was repeated two more times at intervals of 4 days.

Group C consisted of healthy bees, not artificially infected, serving as controls.

Pathohistological examinations showed the first appearance of spores and various stages of N.apis already two days after artificial infection. The Fumagillin therapy gave only a short-term improvement in the health of bees infected with nosema disease.The infective process then continued to progress with a sudden increase in the number of spores in the midgut.

Key words: Nosema apis, pathohistology, fumagillin, therapy

INTRODUCTION

Nosema disease is one of the most wide-spread diseases of adult bees. Investigations by Mladjan et al. (1990) in the region of Zajecar over a period of 4 years showed time -related variations in the presence of nosema disease, but the average prevalence was 50%. Such a significant percentage indicates that the yield of bee products is considerably reduced, as nosema disease essentially shortens the life of bees. Cmejla (1954) found that infected bees never live as long as uninfected ones and that this infection shortens bee life by 25-58%, while Bailey (1976) showed that nosema disease can shorten a bee's life by more than 40%.

Therefore, it is necessary to study the mode and the time of application of medicine. In all investigations performed so far, fumagillin was the only active

matter which yielded satisfactory results (Furgala and Boch, 1970; Van Steenkiste and Jacobs, 1980; Sugden and Furgala, 1979; Popa, 1962). Many authors give advantage to autumn application of fumagillin, but there are also other opinions. Namely, one group of authors believe that it is better to administer fumagillin in spring, at the time when the disease has already developed, which would actually present a therapeutic effect (Grobov, 1971; Poltev, 1971; Peroutka and Veseley, 1976).

In order to study the real therapeutic effects of fumagillin, we caused an infection, applied fumagillin, and then examined midgut sections of bees to detect the first appearance of spores and growth stages of Nosema apis after the infection.

MATERIAL AND METHODS

Healthy (non-infected) young bees (Apis mellifica carnica) from one society were taken from frames with an open brood which included larvae up to 4 days old. Three groups were formed, marked as A, B and C. There were 5 cages with 30 bees each in each group.

Fumagillin DCH, consistsed of the active substance fumagillin and a carrier (Chinoin-Budapest).

Group A - Bees of this group were artificially infected with Nosema apis spores added to sugar syrup (1 ml spores/bee). Bees used up the syrup which contained N.apis spores in 4 days and they were then fed with pure sugar syrup.

Group B - This group served for studies of the therapeutic effect of fumagillin. Bees were infected in the same way as in group A. The bees used up the syrup with N.apis spores in 4 days. After this, they were administered pure sugar syrup, and on the 8th day after infection, bees were given sugar syrup with Fumagillin DCH. During the first administration, there was 0.005 g Fumagillin DCH in 20 ml syrup. The same therapy was repeated two more times within 4-day intervals. The total amount of the applied medicine corresponded to a dose of 4 g Fumagillin DCH per bee society.

Group C - This group of healthy bees was not artificially infected and served as controls. The bees had sufficient quantities of syrup throughout the duration of the experiment.

All three groups were maintained in a thermostat at 31°C, and the bees had drinking water available the whole time of the experiment.

For pathohistological investigations, sections were taken from the midgut of bees of all three groups: 2, 4, 9, 12, 16, 18, and 20 days following infection. Immediately following exenteration, midguts were fixed in 10% neutral Formalin, Bouin and Carnoy solutions. After the usual histological methods and embedding in paraffin, a series of tissue slices were cut on a microtome, 2-6 çm thick, and stained according to Goldner or using the PAS technique.

RESULTS

Group A

Already on day 2 after infection, parasite growth stages and the first spores were detected in certain cells of the midgut epithelium. All growth stages, planonts, meronts, sporonts, and sporoblasts were observed, although the presence of

Figure 1. Midgut. First spores in epithelial cells.

meronts was the most conspicuous. Growth stages were adjacent to nuclei of epithelial cells. The first young spores had a clearly visible vacuole on the poles (Figure 1).

In cross-sections of the midgut the first stages and spores appeared in certain cells of the aboral segment of the midgut, primarily in cells closer to the gut lumen.

Peritrophic membranes were unchanged and their stratification was clearly visible. They were immediately adjacent to the epithelium.

Four days after artificial infection, large numbers of parasite stages and spores were observed in many epithelial cells. In addition to the aboral segment, parasite stages and spores also covered oral segments of the midgut. Desquamation of epithelial cells was also seen, so some preserved desquamated cells filled with stages and spores of N.apis were found in the gut content. In addition to such preserved desquamated cells, we also observed lysed, desquamated cells were so spores released from these cells were present in the

Figure 2. Midgut. Spores without polar filament in gut content (arrow head).

gut content. The presence of young spores was also evident, as well as mature spores from which the polar filament was ejected (Figure 2).

Homogenization of peritrophic membranes was also observed, primarily in the aboral part of the midgut. The stratified structure of peritrophic membranes no longer occurred but they were in the form of wide or narrow homogenous masses.

Preparations obtained 9 days after infection, showed almost all epithelial cells abundant with parasite stages and spores. In most sections, the presence of spores was prevalent in epithelial cells, so that parasite growth stages were difficult to observe. Midgut epithelial cells were taken over by the parasite, from the basal to the apical end. A large number of epithelial cells was pushed out of the epithelial cover and present in the gut lumen. A considerable number of desquamated cells was lysed. A large number of spores and growth stages was released from lysed cells, almost completely filling the gut lumen. The released spores were in the form of bunches, but without any precise order. Young and mature spores were observed, as well as spores from which the polar filament had been released. Peritrophic membranes were homogenized and only their fragments were visible in the aboral area of the midgut.

Twelve days after infection, the epithelial cells were filled with parasite stages and spores, and initial gaps were observed in the epithelium resulting from cell loss (Fig. 3). This epithelial cell loss is due partly to desquamation, and partly to cell lysis. A large number of desquamated cells was observed in the gut lumen,

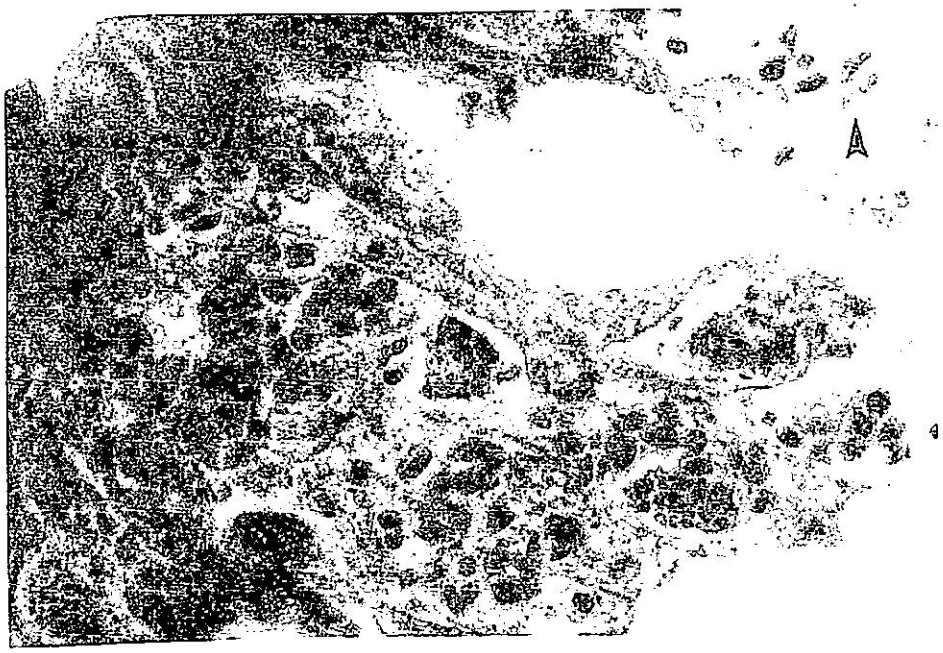

Figure 3. Epithelial cell loss. Sporoblasts (arrow head).

as well as scattered spores which entered the gut content from lysed epithelial cells. The basal membrane was well preserved. No cell activation was seen in regenatory crypts. The circular and longitudinal musculature of the midgut wall was clearly without any changes.

Sections obtained 14 days following infection showed greater changes in the midgut epithelium. The epithelium was scant, so that the gut wall seemed considerably thinner. Parasites were observed in the most distant cells, those lying on the basal membrane.

In addition to the thinning of the gut wall, activation of regeneratory crypts was also observed in certain places. However, cells of regeneratory crypts were not taken over by parasite stages or spores. The basal membrane, and the musculature of the midgut wall were well preserved.

Sixteen days following infection, in certain places the epithelium was visibly reduced to just one row of cells, which were filled with parasite forms of spores. Epithelium loss was observed in places, so that the basal membrane in these places was bare. Penetration of accumulated parasite stages or spores through the basal membrane or musculature of the gut wall was not observed in any preparation. The gut content included a large number of disintegrated epithelial cells form which N.apis stages or spores had been released. N.apis stages or spores were observed in certain epithelial cells of regeneratory crypts (Figure 4). The destruction of degeneratory crypts was also noted.

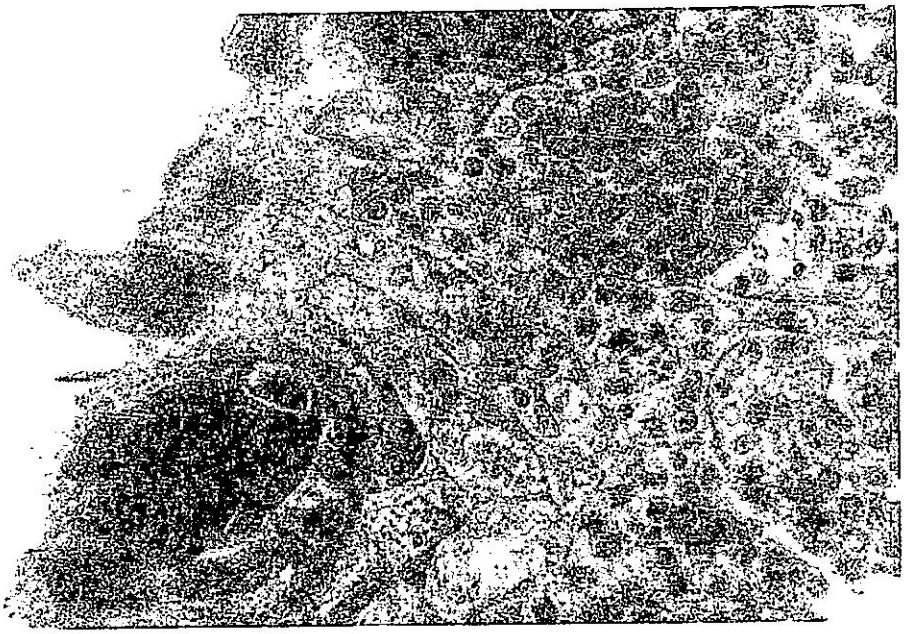

Figure 4. Midgut. Development stages and spores in epithelial cells of regeneratory crypts.

Peritrophic membranes were in the form of fragments, and were not adjacent to the epithelium itself, as a rule, so that there were wider gaps between the gut content and the epithelium.

Group B

Findings were identical to those for group A for all time periods up to 14 days after infection, or 6 days after the 1st and 3 days after the 2nd application of fumagillin.

At 16 days after infection, or 8 days after the 1st and 5 days after the 2nd and 2 days after the 3rd application of fumagillin, there was intensive activation of regeneratory crypts. Newly-formed epithelial cells were clearly visible in certain places as a result of this activation (Figure 5). Consequently, the epithelium was not thinner in all midgut segments. Different stages of parasite development were observed in the newly-formed cells, and less frequently spores. Initial formation of new cells and peritrophic membranes of lamellar appearance was observed in these areas, and even in parts where the epithelium was reduced to a small number of cells.

Examinations of preparations of group obtainedB 18 days after infection, or 10 days after the 1st, 7 days after the 2nd and 4 days after the 3rd application of fumagillin, showed that almost all epithelial cells were filled with parasite stages or spores. Initial gaps in the epithelium resulting from cell lysis were observed. A considerable thinning of the epithelium was seen in certain places. The basal membrane and the midgut wall musculature were well preserved. Peritrophic membranes were homogenized and, in some places, fragmented.

Figure 5. Midgut. Activation of regeneratory crypts.

Figure 6. Midgut. Scant epithelium.

Twenty days following infection, or 12 days after the 1st, 9 days after the 2nd, or 6 days after the 3rd application of fumagillin, significant changes were observed in the midgut epithelium. In some places, it was visibly reduced to just one row of cells which were filled with parasite stages or spores (Fig. 6). Epithelium loss was observed in some places, so that the basal membrane in those places was bare. The basal membrane and musculature were well preserved. The gut lumen was found to contain a large number of lysed epithelial cells from which N.apis stages or spores had been released. Peritrophic membranes were in the form of fragments, and were generally missing from adjacent to the epithelium itself, so that a wider empty space was visible between the gut content and the epithelium.

Group C

Examinations of midgut sections of control bees did not reveal the presence of N.apis growth stages or spores in any sample taken 2, 4, 9, 12, 16, 18 and 20 days after the beginning of the experiment.

DISCUSSION

One of the factors which affect the development of Nosema apis parasites is thr outside temperature. Karmo and Morgengenthaler (1939) reported that parasites grow best and develop spores at temperatures between 30-34°C. Van Laere (1976) was even more precise and gave the optimum temperature for parasite growth as 31°C, which we applied in our investigations. Gray (1968), used a temperature of 36°C, but, like us, observed the first spores in midgut epithelial cells 2 days after artificial infection of bees. These results are the earliest findings of N.apis spores after artificial infection of bees, since Shabanov (1976) and Kovatschev and Shabanov (1972) detected the first spores 3 days after infection, while Bailey (1981) noted spores after 5 days and Revell (1960) 7 days after infection.

Comparing pathohistological findings in the midgut wall between groups A and B in our experiment, we can conclude that differences occurred only 16 days after infection, after the 3 applications of fumagillin in group B. In group A in that period, there were already advanced changes, evident in epithelial loss in certain places of the basal membrane, the penetration of N.apis stages or spores into regeneratory crypts, the destruction of crypts, and, most often, missing peritrophic membranes from along the epithelium. However, in group B, there is a reduced number of infected epithelial cells denoting recovery. Namely, due to the intensive activation of regeneratory crypts, new cells are formed, which are not infected, or are rarely infected with this parasite as a result of the action of fumagillin. Consequently, bare basal membranes were not observed in any places. Peritrophic membranes of lamellar appearance were formed even in those parts of the epithelium where it had been reduced to a small number of cells.

Following such results, we continued to take samples form group B expecting a further reduction of midgut epithelial cell infectiojn in the bees. However, examination of preparations taken 18 days after infection, or 10 days after the 1st, 7 days after the 2nd, and 4 days after the 3rd application of fumagillin, showed a recurrence of infection of almost all epithelial cells in the midgut wall. This is evident as a considerable thinning of the epithelium, and initial gaps are formed due to lysed cells affected by the parasite. Changes took place in

peritrophic membranes in the form of homogenization and fragmentation, which indicated destruction of these morphological components.

By 20 days after infection, or 12 days after the 1st, 9 days after the 2nd, and 6 days after the 3rd application of fumagillin, greater changes were observed in the midgut wall resulting from the effect of N.apis. In fact, the situation was practically identical to that the in group A 16 days after infection. The basal membrane was bare in parts, the epithelium in some places visibly reduced to only one row of cells which were filled with N.apis stages or spores, and peritrophic membranes were fragmented and missing from along the epithelium itself, so that wider gaps were observed between the gut content and the epithelium.

On the grounds of the above, it is evident that therapy with fumagillin actually brings only a brief improvement in bees with nosema disease, and that the infective process then again progresses, perhaps even at a faster rate. Grobov et al. (1967) found that the application of fumagillin prolongs of the life of infected bees, but that the infective process progresses again after termination of fumagillin administration and that there is a sudden increase in the number of spores in the midgut. Our findings also confirm this last observation.

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TERAPIJSKO DELOVANJE FUMAGILINA I PRVA POJAVA SPORA Nosema apis POSLE INFEKCIJE

V. MLADJAN, D. TODOROVIĆ i MIROSLAVA LOLIN

SADRŽAJ

Za sagledavanje terapijskog efekta fumigilina izveden je ogled u kojem je posle veštački izazvane infekcije sa sporama *Nosema apis,* a potom i aplikacije fumagilina izvršen patohistološki pregled isečaka srednjeg creva pčela. Ujedno je praćena i prva pojava spora i razvojnih formi Nosema apis posle izazvane infekcije. U ogled su bile uključene tri grupe pčela:

A grupa -pčele veštački inficirane sporama *N. apis* u šećernom sirupu;

B grupa -pčele kojima je osmog dana nakon inficiranja dat šećerni sirup sa fumagilinom (0,005 g Fumagillina DCH / 20 ml sirupa). Istovetna terapija je ponovljena još 2 puta u razmacima od 4 dana.

C grupa -grupa zdravih pčela koja nije bila veštački inficirana,

Patohistološkim pregledom je utvrđena prva pojava spora i razvojnih formi *N. apis* već 2 dana posle veštačkog inficiranja. Terapija fumagilinom je samo kratkotrajno uticala na poboljšanje zdravstvenog stanja pčela obolelih od nozemoze. Posle terapije infektivni proces ponovo napreduje i dolazi do naglog porasta broja spora u srednjem crevu pčela.