Full Length Research Paper

Evaluation of oxytetracycline in the prevention of American foulbrood in bee colonies

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In this paper, we presented the procedure of artificial infection of bee colonies with American Foulbrood by the spores of Panibacillus larvae (P. larvae). Bee colonies were treated with oxytetracycline that was applied in a patty and sugar syrup. The symptoms and the course of the disease within the bee colony were monitored. Our research included 20 bee colonies divided in three experimental and one control group. Every bee colony consisted of 2000 young worker bees and a young breeder queen. After laying of first eggs three experimental groups were infected with spore-suspension of P. laervae at concentration 2x10⁹ spore/ml. The development of the disease was monitored under the following experimental conditions: Experimental group I did not receive antimicrobial therapy, Groups II and III were given antibiotics in a sugar syrup and extender patty, respectively. In the first experimental group the first clinical signs were observed on day 25, while vegetative form of P. larvae was detected on day 12. Treatment with oxytetracycline in the second and third group delayed the manifest disease until day 47.

Key words: Bee colonies, American foulbrood, oxytetracycline.

INTRODUCTION

American foulbrood is one of the most serious and destructive diseases of honeybee larvae that imperceptibly enter hives, spreading slowly and persistently, and finally resulting in the death of bee colony. In epizootiological sense American foulbrood is a typical chain-infection, one of the most widespread diseases of the bee brood (Đuričić et al., 2001). The causative agent is a bacterium Panibacillus larvae (Heyndrickx et al., 1996). It mostly affects the larvae of working bees, but it is contagious also for larvae of the drone and queen bees. Adult bees are not susceptible. P. larvae is apparent in two forms, the vegetative form (rods) and the form of spore, the latter one being infective for honeybees. Bee larvae less than 53 h old become infected orally, by swallowing P. larvae spores contained in their feed. The older larvae are not susceptible. Spores are rarely detectable in larvae more than 53 h old. The spore germinates into the vegetative form immediately after entering the digestive tract of the larva, continuing to multiply until larval death. After the larva dies, new spores are formed. In only one cell with the dead larva as much as 2.5 milliard spores can be found (MSDTS OIE, 2000).

The number of spores required to produce the infection varies depending on other factors. Sturtevant and Revell (1953) produced infection using 5x10⁷ spores of P. larvae/g honey, while Hansen and Brodsgaard (1995) observed that feeding the bee colony with honey containing 2x10⁹ spore/g honey inevitably caused the outbreak of American foulbrood.

In the pathogenesis of the disease, it is well established that spore of P. larvae introduced into the organism of a young larva in opened brood does not immediately germinate into its vegetative form; however, it remains for a while in the intestine of the larva. After covering of cells,
Antibiotics, applied in the feed, affect the course of the disease. For this investigation bee colonies free from American foulbrood were chosen. Examination of the honey from the investigated hives revealed absence of *P. larvae* spores; hence no antimicrobial therapy was applied. "Pin-test" of the colony revealed high hygiene instinct in bees.

**Microorganisms**

Artificial infection was performed using referent strains of *P. larvae* (5875 CAMP, Collection of Animal Pathogenic Microorganisms, Veterinary Research Institute, Czech Republic).

**Culture media**

A special nutritive culture medium J-agar was used, consisting of: 20 g agar, 5 g tryptone, 15 g yeast extract, 3 g K$_2$HPO$_4$, 2 g glucose and one liter of demineralized water; blood agar (nutritive agar with 8% defibrinated sheep blood), nutritive medium with 2% NaCl, and nutritive medium with 5% NaCl, nutritive broth adjusted at 6.8 pH.

**Antibiotics**

"Vazemicin R" ad. us. vet. was used, containing 55 mg oxytetracycline/1 g of powder for oral application (producer: Veterinary Institute, Zemun).

**Study subjects**

This study included 20 bee colonies distributed in three experimental and one control group. Each colony consisted of about 2000 young worker bees and a young breeder queen. All experimental groups were infected with *P. larvae* spore suspension, at concentration 2x10$^9$ spore/ml. The course of the disease was monitored under the following conditions:

Experimental group I did not receive the oxytetracycline therapy.

Experimental group II was given oxytetracycline at concentration 0.1 g/100 g of sugar syrup. The therapy was applied during 30 days, at 48-h-intervals (in the evening). Experimental group III received oxytetracycline in the extender patty at concentration 0.1 g/100 g. Clinical and laboratory examinations were performed on days 12, 25, 47 and 68. (Table 1)

**RESULTS**

Microscopic examination performed on day 12 revealed the presence of vegetative rods of *P. larvae* in two colonies of the Experimental group I. Clinical examination on day 25 confirmed symptoms of American foulbrood in the same group, that is, overall comb appearance was "patchy" (Figure 2), cappings were dark in color (Figure 1) and larvae were colored chocolate-brown, elastic and

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Table 1. Plan of the trial and situation in different phases.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental group (O-I)</th>
<th>Experimental group (O-II)</th>
<th>Experimental group (O-III)</th>
<th>Control group (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of hives</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Size and power of the colony</td>
<td>2.000 bees + young breeder queen</td>
<td>2.000 bees + young breeder queen</td>
<td>2.000 bees + young breeder queen</td>
<td>2.000 bees + young breeder queen</td>
</tr>
<tr>
<td>Type of feed</td>
<td>sugar syrup 1:1</td>
<td>sugar syrup 1:1 + OTC</td>
<td>Extender patty + OTC</td>
<td>extender patty</td>
</tr>
<tr>
<td>Quantity of feed per colony</td>
<td>10 ml/da</td>
<td>10 ml sugar syrup +0.1 g OTC/at 2-day intervals</td>
<td>100 g extender patty + 0.1 g OTC/ at 5-6-day intervals</td>
<td>100 g extender patty/ at 5-6-day intervals</td>
</tr>
<tr>
<td>Situation on day 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control on day 12</td>
<td>No change in the comb appearance</td>
<td>No change in the comb appearance</td>
<td>No change in the comb appearance</td>
<td>No change in the comb appearance</td>
</tr>
<tr>
<td>Bacteriological control and finding</td>
<td>Bacterial rods in 2 colonies; Spores of <em>P. larvae</em> in 3 colonies</td>
<td>Negative finding</td>
<td>negative finding</td>
<td>-</td>
</tr>
<tr>
<td>Situation on day 25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comb appearance</td>
<td>&quot;patchy&quot; comb, seals dark in Color, larvae of chocolate color and elastic</td>
<td>Compact, convex cappings; in one colony cappings darker in color</td>
<td>Compact, convex capping</td>
<td>Compact, convex capping</td>
</tr>
<tr>
<td>Bacteriological control and finding</td>
<td>Bacterial rods of <em>P. larvae</em> in the larvae in 2 colonies; spores of <em>P. larvae</em> in 1 colony</td>
<td>Negative finding</td>
<td>Negative finding</td>
<td>Negative finding for the examined sugar syrup</td>
</tr>
<tr>
<td>Situation on day 47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comb appearance</td>
<td>&quot;Patchy&quot; comb, seals dark in color, larvae of chocolate color and elastic</td>
<td>&quot;Patchy&quot; comb, seals dark in color, larvae of chocolate color and elastic</td>
<td>&quot;Patchy&quot; comb, seals dark in color, larvae of chocolate color and elastic in three colonies</td>
<td>Compact, convex cappings</td>
</tr>
<tr>
<td>Bacteriological control and finding</td>
<td>Bacteria rods of <em>P. larvae</em> in 1 colony; spores of <em>P. larvae</em> in 3 colonies</td>
<td>Spores of <em>P. larvae</em> in 2 colonies, bacterial rods of <em>P. larvae</em> in 3 colonies</td>
<td>negative finding</td>
<td>negative finding</td>
</tr>
<tr>
<td>Situation on day 68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comb appearance</td>
<td>-</td>
<td>&quot;Patchy&quot; comb, seals dark in color, larvae of chocolate color and elastic</td>
<td>&quot;Patchy&quot; comb, seals dark in color, larvae of chocolate color and elastic in two colonies</td>
<td>Compact, convex cappings</td>
</tr>
<tr>
<td>Bacteriological control and finding</td>
<td>-</td>
<td>Spores of <em>P. larvae</em> in the colony</td>
<td>Spores of <em>P. larvae</em> in both colonies</td>
<td>Negative finding</td>
</tr>
</tbody>
</table>
examination revealed presence of bacterial rods in the Experimental group II.

Clinical and bacteriological examinations performed on day 47. revealed the American foulbrood in all three experimental groups. In the Experimental groups I and II two and three bee colonies were alive, respectively. In the Experimental group III all bee colonies were alive. In the control group no changes were observed, and all the colonies were alive.

On day 68. no more living colonies were observed in the Experimental group I, while only one living colony in the Experimental group II that exhibited pronounced clinical signs of the disease. In the Experimental group III we observed 2 living bee colonies with marked symptoms of the American Foulbrood and positive bacteriological findings.

DISCUSSION

American foulbrood is a persistent, progressive, contagious disease of the honeybee brood. The infection appears in closed broods, and the outcome is always lethal. In our country the disease is widely spread and under Serbian health legislation it is notifiable and must be reported to the relevant authorities. In recent years antibiotics are increasingly applied in the "treatment and control" of the American foulbrood.

The susceptible larva is essential for the infection; however, development of the disease is determined by the number of $P.\text{larvae}$ spores. The number of spores varies depending on different factors. Sturtevant and Revell (1953) induced artificial infection with American foulbrood with $5 \times 10^7$ spores of $P.\text{larvae}/g$ honey, while Hansen and Brodsgaard (1995) observed that feeding bee colonies honey containing $2 \times 10^9$ spore/g honey necessarily caused the outbreak of the disease. Feeding of the larvae during the first six days, while the cell is still open, is very intensive. It is assumed that young larvae at this stage are visited and fed by feeding bees about
8,000 times, enlarging their weights 100 times during the first 3 days, and even 500 times within 6 days, which is the unique event in animal kingdom (Tomašec, 1955). Such a frequent feeding may easily introduce certain number of *P. larvae* spores into the larva. The least infective dose necessary to cause the infection is 35 spores for one-day-old larva (Woodrow, 1941); however, investigation performed next year by the same author revealed that only 10 spores are sufficient for one-day-old larva, and millions of them are needed to induce the infection in 4 to 5-days-old larvae. Brodsgaard (2003) indicated that the larvae are most susceptible between 24 and 28 h of age, with the lowest required infective dose being (ID$_{50}$) 8.49 per one larva.

Our results indicated that in the Experimental group I, where artificial infection with *P. larvae* was performed, first clinical symptoms of American foulbrood and the first spores were detected after 25 days. In the Experimental group II that was treated with oxytetracycline in sugar syrup first signs of the disease were postponed until day 47. In the Experimental group III treated with oxytetracycline in extender patty American foulbrood was clinically detected on day 47, but all the colonies were alive, and one of them survived even 68 days.

Similar results are reported by Oldroyd et al. (1989), who monitored the effects of oxytetracycline on 20 infected bee colonies. Colonies were housed in LR (eight framed) beehives and each colony was infected with 2×10$^{5}$ spore per 1 ml of suspension.

It was discovered that 1 g oxytetracycline applied at the time of inoculation with *P. larvae* spores might postpone the disease in the hive (in two colonies) for 29 to 87 days, and in other colonies even up to one year. The same authors concluded that treatment with oxytetracycline in sugar syrup may delay American foulbrood for about one month, but application of oxytetracycline as prophylactic measure is not recommended. The results obtained in our study suggest that short period of delay is most probably due to the relatively small number of bees. Namely, our experiment included about 2000 bees, which is relatively small number in comparison to colonies in LR-beehives (extenders), with approximately 40,000 to 50,000 bees.

Similar results are reported by Baillay (1981), who observed that application of oxytetracycline in concentration of 0.25 to 0.40 g in 5 l sugar syrup per infected colony was not effective, while mixture of oxytetracycline, herbal fats and sugar, so-called "grease patty" was effective.

Application of antibiotics in apiary practice is of recent date. Chemotherapy was applied in some countries as early as in 40's of the 20th century, and sulpathiazole was considered a "miraculous remedy". Some years later, in the 50's, oxytetracycline was introduced. Numerous reports on research in this field were published (Lolin et al., 1987; Shimmanuki, 1997; Allipi, 1999), and it was well established that oxytetracycline and other antibiotics did not affect the spores of *P. larvae*, impacting only its vegetative form that is present at the beginning of the infection. This indicates that only continual antimicrobial therapy could protect bee colonies, which on the other hand, caused resistance of *P. larvae* to oxytetracycline. Reports of Shumanuki (1997) confirmed the resistance in Argentina, America and Canada. Chemical analyses of honey samples revealed presence of oxytetracycline residues that are highly harmful for human health (Mckee, 2003; Plavša et al., 2003; Grujić 2003).

REFERENCES


