Introduction

Foulbrood diseases rank among the most dangerous of the known bee colonies pathologies. American foulbrood is one of the most widespread bee brood diseases\(^1\),\(^2\). Currently this disease often manifests itself in the form of mixed infection. Coincident with American foulbrood they register European foulbrood, parafoulbrood (up to 65%) and chalk brood. Manifestation of American foulbrood in the form of mixed infection complicates its course and changes clinical signs of its manifestation\(^3\).

Epizootological surveys of apiaries of different regions of Ukraine allowed to establish frequency and form of this disease manifestation. Microbiological examination of comb honey from clinically healthy bee colonies demonstrated rather frequent presence of pathogens of the above-mentioned diseases. At the same time such bee colonies not always have clinical manifestation of a disease in the course of a season. Epizootology explains this fact, as a disease development necessitates a number of factors: sufficient concentration of spores in food, lowering of activity of factors of natural resistance of bee larvae etc.

At present bee infectious diseases are extensively controlled with antibiotics and chemical preparations that contaminate beekeeping products and not always ensure high curative effect due to pathogen resistance development.

The aim of our study was the developing and testing of an alternative method of prophylaxis and treatment of bee brood infectious diseases.

Materials and Methods

Our experiment involved 80 bee colonies of the Ukrainian steppe race. The experiment lasted from 10.05 till 30.06.1999. The bee colonies were subdivided into four groups of 20 bee colonies each. 10 bee colonies manifested clinical signs of mixed infection (American foulbrood and chalk brood – up to 10 dead larvae per family) and 10 bee colonies were conventionally healthy, i.e. clinical signs of a disease were not revealed but comb honey contained viable spores of \textit{P. larvae} and \textit{Asc. apis}. Varroosis invasion degree of bee colonies at the beginning of an experiment was within 0.9-1.9%.

Bee colonies of the first group were treated four times with curative and prophylactic purpose with oxytetracycline hydrochloride in the dose of 400000 U per 1 l of syrup with 5-6 d interval.

Bee colonies of the second group were fed sugar syrup with “Apitonus” biological preparation (animal blood hydrolysis product). They were fed 4 times in the dose of 8 ml per 5 l of syrup by 1 l per family with 5-6 d interval.

Bee colonies of the third group were administered sugar syrup with a vaccine against American foulbrood. The vaccine was administered 4 times in the dose of 80 ml per 1 l syrup by 1 l per family with 5-6 d interval.

The bee colonies of the fourth group were fed 1 l pure sugar syrup per family within the same time and interval range.

Combs were disinfected with water solution that contained 0.5% of the “Devosan forte” preparation (an active substance – stabilized peracetic acid), 10% of hydrogen peroxide and 5% of the “Brovadez-10” preparation (benzalkonium chloride).

The disinfectants were applied in compliance with the previously developed modes of disinfection. Treatment exposition equalled to 5-8 h at 18-20 °C.

Bacteriological and mycological examinations of bee brood, honey and comb washings aimed at detection of viable pathogens of bacterial and fungal bee diseases were carried out in compliance with the previously developed methods.

All bee colonies were examined in 30 days after treatment completion. Honey and brood were sampled for examination of presence of viable infectious disease pathogens.

To expand brood nest during test period they used disinfected comb frames only or set foundation to build new frames. Frames with dead larvae were set aside to the nest edge and isolated with a separation grid till the complete brood emergence and set back to the nest after disinfection. Beehives were cleaned and disinfected with 0.5% solution of Devosan forte or 5% Brovadez-10.

Bee varroosis affection degree was monitored during all test period. Treatments were carried out twice in a season: in summer, after extraction of honey (5-10.08) and in late autumn, after complete brood
emergence. Treatment was carried out with acaricide-impregnated thermal strips (Tactic, 0.125 g per a strip). Preparation was applied two times at the rate of one strip per 10 frames with bees.

To replenish winter food store they used sugar syrup with the “Apitonus” preparation admixed in the dose of 8 ml per 10 l of syrup. Each bee colony was supplied with 5-8 kg of sugar as a syrup at the end of a season.

**Test results**

The results of practical testing of the developed curative and prophylactic bee colony treatment schemes at mixed forms of American foulbrood are presented in Table I.

<table>
<thead>
<tr>
<th>Bee group</th>
<th>Test start</th>
<th>Test finish</th>
<th>Test start</th>
<th>Test finish</th>
<th>Test start</th>
<th>Test finish</th>
</tr>
</thead>
<tbody>
<tr>
<td>American foulbrood diseased</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>2</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Conventionally healthy</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

As the table shows, high curative and prophylactic effect was achieved in the first group of oxytetracycline-treated bee colonies. The diseased group had no bee colonies with clinical symptoms by the end of the test period and all bee colonies within the conventionally healthy group remained healthy.

Eight of the diseased bee colonies of the Apitonus treated group had no clinical signs and two remaining colonies had solitary dead larvae (1-3 per family). All conventionally healthy bee colonies preserved clinical health.

Maximum curative and prophylactic effect was also observed within the bee colony group that was treated with the vaccine against American foulbrood. All clinically ill bee colonies had no disease signs by the end of the test period and conventionally healthy colonies preserved clinical health.

9 of 10 bee colonies of the control group that were fed pure sugar syrup preserved overt clinical signs of foulbrood (up to 5-10 dead larvae per family). In five beehives of the conventionally healthy bee colony group there were detected solitary dead larvae (1-4 per family) with typical foulbrood signs and solitary mummified larvae. After clinical examination of all bee colonies of experimental and control groups comb honey was selectively sampled and tested for presence of viable infectious disease pathogens. The results are presented in Table II.

<table>
<thead>
<tr>
<th>Bee group</th>
<th>1999</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. larvae</td>
<td>Asc. apis</td>
</tr>
<tr>
<td>First test group (oxytetracycline)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Second test group (Apitonus)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Third test group (vaccine)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Control group (sugar syrup)</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

The table shows that all samples of honey combs of the test and the control bee colonies were contaminated with viable spores of American foulbrood and chalk brood pathogens.

Bee colonies of the control group were additionally treated with curative syrup that contained oxytetracycline to prevent disease spreading in the apiary in compliance with the recommendations in force.

The results of seasonal test colony observation were indicative of the absence of recurrence cases.

Presence of viable pathogen spores in food store plays a significant role in the appearance of bee brood infectious disease. To remove this factor from epizootic chain total honey store was extracted from the above-mentioned bee colonies at the end of a season and food store was replenished with sugar syrup. When replenishing food store they fed sugar syrup with Apitonus addition at the rate of 8 ml per 10 l of syrup.

Colonies were ready for overwintering stay after food store replenishment, nest formation and carrying out of final varroosis treatment complex.

Overwintering was successful, all bee colonies were alive at the moment of flight (6.03.2000). The first, second and third bee groups were integrated in spring and the 3 one remained unchanged. All bee colonies were examined, unnecessary empty frames were removed from the nests and a nest was contracted in order all frames to be occupied by bees. Bee colonies of the first, second and third group were fed sugar syrup as a stimulative feeding with the Apitonus addition at the rate of 8 ml per 5 l of syrup.

Bee colonies of the third group were administered a vaccine against American foulbrood in the above indicated doses. Apitonus and vaccine were administered 4 times with 5-7 days interval.
In spring time, as the colonies were built-up, the brood nest was expanded through the utilization of disinfected frames and by artificial foundation building.

The results of observation of bee colonies of all groups during the season of the year 2000 are indicative of their sanitary welfare in respect to American foulbrood and chalk brood.

Tests of selective honey comb samples of all bee-family groups demonstrated presence of viable spores of American foulbrood and chalk brood pathogens (Table II). At the same time there was no clinical manifestation of a disease during the season.

Discussion

Nowadays many researchers undertake attempts to breed a bee line with high hygienic behaviour level at brood infectious diseases and produce probiotics that can inhibit the development of pathogenic microflora under natural conditions[4].

Our experiments were aimed at the development of methods of enhancement of activity of natural bee resistance factors at unfavourable epizootic situation. When developing methods of prophylaxis and control of different manifestation forms of American foulbrood we resorted also to the methods of deactivation of pathogen spores and the reduction of its number in a colony. With this aim in view, disinfectants with the highest preliminary tested activity were used.

As the observation of long standing shows, this method permits of epizootic chain breakage at this disease and ensures high efficiency. The developed method efficiency is highly competitive with the conventional antibiotic therapy. At the same time it allows us to get ecologically pure beekeeping products without antibiotic residues.

Moreover, application of specific and non-specific biological preparations (vaccine against American foulbrood and Apitonus) has a marked enhancing impact on the development of a colony as a whole.

The recommended method has been practically approved on apiaries of the Ukraine and is extensively used by apiarists.

REFERENCE