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RESEARCH ARTICLE

Evaluating Bactericidal Effect of the Antibiotics on the European Foulbrood Disease in Honeybees

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Abstract

The paper discusses the results of testing the bactericidal and bacteriostatic effect of various concentrations of pefloxacin and enrofloxacin on European foulbrood pathogens in contrast to oxytetracycline. The work presents the therapeutic efficacy of pefloxacin in treating bee colonies affected by European foulbrood disease. The study tested different methods of introducing medicine in the field experiment. European foulbrood disease revealed at the studied apiaries is caused by Melisococcus plutonium, Bacillus alvei, and Enterococcus faecalis; Brevibacillus laterosporus. In Nurimanovsky and Baimaksky districts (Republic of Bashkortostan, Russia), Melisococcus plutonium, which is the primary causative agent of EFB, has not been detected. The minimum bactericidal concentration of pefloxacin is 0.001% for Enterococcus faecalis, while the minimum level for Brevibacillus laterosporus and Bacillus alvei is 0.01%. Oxytetracycline exceeds the index 10fold, and it is 0.01 % for Enterococcus faecalis and 0.1 % for Brevibacillus laterosporus and Bacillus alvei. The minimum bactericidal concentration of pefloxacin is 0.01% for Melisococcus plutonium, which is 100 times lower than the minimum concentration of oxytetracycline (1.0%). High therapeutic efficacy is achieved (93.8...95.9%) in treating bee colonies affected by European foulbrood disease with pefloxacin by adding the antibiotic to the feed syrup (1:1) at a dose of 0.01%. The value is significantly higher than the effect of oxytetracycline applied at a dose of 0.05% (87.4...88.2%).

Keywords: *Apis mellifera mellifera L,* European foulbrood disease, Bactericidal activity, Fluoroquinolones, pefloxacin.

Introduction

European foulbrood disease is a disease occurred in honeybees and affecting young larvae. The larvae affected by EFB tend to change their colour from white to grayish-black, smell foul, and, as a result, die before pupation [1]. *Melissococcus plutonius* is the primary causative agent of European foulbrood desease [2]. *Achromobacter eurydice*, *Bacillus pumilus*, *Brevibacillus laterosporus*, *Enterococcus faecalis*, *Paenibacillus alvei*, *Paenibacillus dendritiformis* make up the secondary invading microflora [3-5]. The disease is widespread all over the world, being the main reason for the mass collapse of bee colonies [6, 7]. Therefore, researchers from different countries are engaged in searching for methods to prevent and treat the disease.

Some researchers have established the antagonistic effect of lactic acid bacteria, and some strains of the *Bacillus* genus from the gut of *Apis mellifera* and *Apis cerana* against *M. plutonius* [8, 9]. Studies have revealed a specific beneficial effect of the preparation TANG containing *Barilla Licheniformes* [10]. However, antimicrobial peptides and low pH of royal jelly tend to inhibit the growth of these bacterial strains and reduce the positive effect of probiotics in the treatment of European foulbrood disease [9]. A 2% solution of royal jelly produces an inhibitory effect on not only the secondary microflora but also the EFB principal causative agent *Melisococcus plutonium* [11]. The researchers point out that the effect can be accounted for by the activity of MRJPs protein as the main component of the royal jelly. The study isolated antimicrobial material from the bee larvae affected by ascespherosis. The material could inhibit the growth of *P. larvae* and *M. plutonius in vitro*. They identified the ma-

terial as fatty acids. On the whole, the researchers have isolated a total of 28 antimicrobial substances. Fifteen of the isolated substances had an inhibitory effect on *P. larvae*, and eight of the substances affected *M. plutonius* [12]. However, the computational study of EFB pathogen transmission on the laboratory-grown larvae showed that these acids failed to protect against infection [13, 14]. The researchers pointed out that the discrepancy resulted from the apparent inactivation of fatty acids by larval feed components or the gut microflora.

Trials on antibiotics for EFB treatment have shown that penicillin has low effectiveness against the disease, while erythromycin and oxytetracycline are relatively effective [15]. *M. pluton* is highly sensitive to ampicillin and amoxicillin. However, these antibiotics are used in medical applications, and they can cause allergic reactions in sensitive humans. Excessive use of ampicillin, amoxicillin, and oxytetracycline may result in contaminated honey and other apiary products [15].

Currently, the active drug oxytetracycline is the recommended and most common treatment against foulbrood bacteria at the apiary [7, 16, 17]. Nonetheless, the larval foulbrood disease remains urgent mainly due to the polymicrobial aetiology of the disease and relatively high resistance of pathogens, as well as the growing resistance of the pathogens to antibiotics [3, 6, 18]. Therefore, it is relevant to identify new and effective methods of treatment and prevention of European foulbrood disease. The present study focused on the following: evaluating the possible application of two fluoroquinolone antibiotics pefloxacin and enrofloxacin to treat and prevent the disease, determining the sensitivity of pathogens to the antibiotics; identifying the therapeutic effect of the drugs.

Materials and Methods

The laboratory study involved the following steps: the combs with brood were examined for clinical symptoms of the diseased larvae; if detected, 10x15 cm comb samples with dis eased and dead larvae were taken for analysis. Ten to fifteen dead larvae or their remains were removed from the comb cells with sterilised forceps; then the material was placed in a mortar which was filled with 5 ml of sterile saline solution, the material was then mixed vigorously until a homogeneous suspension was obtained. The obtained pathological material was placed in meat-peptone broth (MPB) and on a meat-peptone agar (MPA) plate. The Cherepov's medium was formed for culturing *Melisococcus* plutonium. The spread-plated material was examined following 4-5 day incubation. *Melisococcus plutonium* forms round, small (1.0 -1.5 mm in diameter) convex granular colonies of pearl-white color. Conventional procedures were used to identify *Enterococcus faecalis, Bacillus alvei, and Brevibacillus laterosporus* based on morphological, tinctorial, and biochemical properties. Pure bacterial cultures of all pathogens detected at each apiary were then subcultured from the grown colonies.

In order to examine the minimum antimicrobial concentration of pefloxacin, enrofloxacin, and oxytetracycline, the study used liquid broth minimum inhibition assay. Sterile MP broth was prepared in paillettes, solutions of the selected antibiotics were added by successive serial dilution. As a result, solutions with 10, 1, 0.1, 0.01, 0.001, 0.0001 and 0.00001% concentrations were obtained. At the same time, the study monitored the growth of pathogens in the medium without antibiotics. Next, a suspension of microbial cells was made from a pure bacterial culture of each pathogen; the suspension was then inoculated into paillettes. The study recorded the bactericidal effect of the tested antibiotics daily. The study identified antibiotic concentrations that did not produce growth in colonies (no turbidity or sediment) after 72 hours (for anaerobes-after six days) as bactericidal.

Concentrations that inhibited the growth of cultures for 48-72 hours (for anaerobes-for 5-6 days) were considered bacteriostatic. Disc diffusion assay and agar-based serial dilution tests were used to estimate the sensitivity of the bacterial pathogens isolated from the pathological material to the tested antibiotics *In-Vitro.*For the disc diffusion assay discs with antibiotics in 10, 1, 0.1, 0.01, 0.001, 0.0001 and 0.00001% concentrations were prepared. Just before the test, Petri plates were filled with a dense nutrient medium for culturing microorganisms. So the test used the Cherepov's medium for *Melisococcus plutonium* and 3 % glucose MPA for the rest of the EFB pathogens. The discs with the tested antibiotics were placed on the nutrient media no later than 15 minutes after seeding the pathogen cultures. Simultaneously, the study monitored the growth of pathogens in the media by application of the discs without an antibiotic.

The plates were incubated in a thermostat immediately after the disc application to exclude the antibiotics pre-diffusion. Following incubation, the diameter of growth inhibition zones was measured with an accuracy of 1 mm. The analysis implied that the absence of a sterility zone indicated the resistance of the pathogen to the antibiotic concentration, the diameter of the growth inhibition zone of up to 14 mm indicated low sensitivity, a 15 to 25 mm diameter indicated sufficient sensitivity. A diameter of over 25 mm showed high sensitivity of the pathogen to a given concentration of antibiotics.

The agar-based serial dilution test involves seeding microorganisms on Petri plates with the agar containing successive dilutions of antibiotics. A series of 10 ml paillettes with 10, 1, 0.1, 0.01, 0.001% and 0.0001% dilutions of the tested antibiotics were pre-prepared. First, the antibiotic solution was put in the plate, then the agar heated to 50ºC was added. After careful mixing and uniform distribution of the antibiotic in the medium, the plates were left to solidify and dry for 10-12 hours.

The pure bacterial culture of each pathogen was inoculated on the agar plate by a bacteriological loop in the center of the medium and slightly distributed by circular movements of the plate. After drying, the plates were placed in a thermostat. The test results were examined by placing the plate on a dark surface that does not reflect light. The study considered the concentration of antibiotic that caused complete visible growth inhibition of the culture as the bactericidal concentration.

At the same time, the study monitored the growth of pathogens in the medium without antibiotics. The apiary based experiment used four groups of bee colonies formed on the pair-analog principle. The analysis studied the strength of the bee colony, the number of combs in the hive, the presence and the age of the bee queen, the number of EFB diseased larvae, the number of dead larvae or their remains (putrid mass, dried pupa).

Two methods of introducing pefloxacin into bee colonies were tested: feeding as part of the larval diet (Group 1) and spraying with a medicinal solution (Group 2). Oxytetracycline was introduced only with feed (group 3). Bee colonies of the control group received no antibiotics. The experiments used such a minimum concentration of antibiotic to which the most resistant EFB pathogen had sufficient sensitivity, and which was able to inhibit the growth of all pathogens of the disease for more than five days after the incubation in the laboratory.

The drug solution for groups 1 and 3 was prepared by diluting the antibiotic in 50 ml (35-40°C) warm water; the solution was then mixed with 10 l sugar syrup (1:1). The Medicinal syrup was given to bees twice at 2-3 days intervals. The syrup was put into the upper feed boxes at the rate of 100-120 ml per comb. For group 2, the solution was prepared by mixing the drug with 10 liters of sugar syrup (1:4). Treatment involved spraying the combs with bees and brood daily for three days, at the rate of 10-15 ml per 1 comb.

The bees of group 4 received sugar syrup without drugs. The control of the drug efficacy included a visual examination of the brood before the treatment and 6, 9, and 12 days after the treatment and laboratory analysis of brood samples from the hives. The number of diseased larvae was counted every three days. The experiment calculated the extent of the diseased brood before and after the experiment.

Results

For the study, samples of the pathological material were collected from the bee colonies that demonstrated EFB symptoms. Several districts of the Republic of Bashkortostan (Russia) provided their apiaries for the study. The study revealed EFB pathogens in different associate variants (Table 1).

Table 1: FB pathogens identified in the districts of the republic of bashkortostan

District	FB pathogens revealed				
	Mel. plutonium	***Bac. alvei***	***Ent. faecalis***	***Brevibac. laterosporus***	***Paenibac. larvae***
Aurgazinsky	+	+	+	-	+
Baymaksky	-	+	+	-	-
Beloretsky	+	+	+	-	-
Kugarchinsky	+	+	+	-	-

Nurimanovsky	-	+	+	+	-
Ufimsky	+	+	+	-	+

"+" sign denotes the presence of the pathogen in the pathological material.

Samples taken from the apiaries of Baymaksky, Kugarchinsky, and Nurimanovsky districts revealed EFB pathogens of *Melisococcus plutonium, Bacillus alvei*, and *Enterococcus faecalis.* For the first time, the study revealed *Brevibacillus laterosporus* as part of the secondary microflora in the pathological material taken from the apiary of Nurimanovsky district.

The pathological material from apiaries of Aurgazinsky and Ufimsky districts contained EFB pathogens (*Bacillus alvei, Enterococcus faecalis, and Melisococcus plutonium*) in combination with American FB pathogen (*Paenibacillus larvae).* Special mention should be made of the apiaries in Baymaksky and Nurimanovsky districts. The study could not isolate *Melisococcus plutonium* in the pathological material taken from the apiaries, though the colonies from where samples were collected demonstrated all symptoms of FB disease. *Bacillus alvei* and *Enterococcus faecalis* were the most common among secondary invaders in all of the districts. In order to estimate the sensitivity of the pathogens to antibiotics pure bacterial cultures of EFB pathogens were isolated from the pathological material taken from the apiaries in Aurgazinsky district. The selected antibiotics were oxytetracycline (OTC) used for EFB treatment in beekeeping, pefloxacin, and enrofloxacin being two antimicrobial agents of the fluoroquinolone series. In order to identify the minimum bactericidal concentration (MBC), the study used the test of serial dilutions in meat-peptone broth (MPB). In essence, the successive 10-fold dilutions were made.

The experimentation showed that *Melisococcus plutonium bacteria had the lowest sensitivity to the selected antibiotics.* For complete inhibition of the pathogen growth, the experiment required higher drug concentrations than for the inhibition of other isolated microorganisms: of pefloxacin - 0.01%, of enrofloxacin-0.1%, and OTC-1% (Table 2). For the analysed pathogen, the bacteriostatic concentrations were as follows: of pefloxacin - 0.001%, enrofloxacin -0.01%, and OTC -0.1%. At the given concentrations, pefloxacin and oxytetracycline inhibited bacterial growth for 24 hours, enrofloxacin - for 48 hours.

Table 2: Bacteriostatic and bactericidal concentrations of the tested antibiotics against EFB identified by the method of serial dilutions in MPB

Antibiotics	Antibiotic concentration,%	The pathogen culture growth											
		Time after inoculation, day.											
		2	3	4	5	2	3	4	5	2	3	4	5
		Bac. alvei				*Ent. faecalis*				*Mel. plutonium*			
Pefl	10	-	-	-	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-	-	-	-
	0.1	-	-	-	-	-	-	-	-	-	-	-	-
	0.01	-	-	-	-	-	-	-	-	-	-	-	-
	0.001	-	-	-	+	-	-	-	-	-	+	+	+
	0.0001	+	+	+	+	-	+	+	+	+	+	+	+
	0.00001	+	+	+	+	+	+	+	+	+	+	+	+
Enrofl	10	-	-	-	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-	-	-	-
	0.1	-	-	-	-	-	-	-	-	-	-	-	-
	0.01	-	-	-	-	-	-	-	-	-	-	+	+
	0.001	-	+	+	+	-	-	+	+	+	+	+	+
	0.0001	+	+	+	+	+	+	+	+	+	+	+	+

	0.00001	+	+	+	+	+	+	+	+	+	+	+	+
OTC	10	-	-	-	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-	-	-	-
	0.1	-	-	-	-	-	-	-	-	-	+	+	+
	0.01	-	-	-	+	-	-	-	-	+	+	+	+
	0.001	-	-	+	+	-	+	+	+	+	+	+	+
	0.0001	+	+	+	+	+	+	+	+	+	+	+	+
	0.00001	+	+	+	+	+	+	+	+	+	+	+	+

OTC stands for oxytetracycline, Pefl stands for pefloxacin, Enrofl for enrofloxacin; '+' sign is for the presence of visible growth, '-' is for no visible growth.

For *Bacillus alvei*, the same concentrations of pefloxacin were bactericidal and bacteriostatic as for *Melisococcus plutonium*. Enrofloxacin had a bactericidal effect at a 0.01 % concentration, and a bacteriostatic effect at 0.001% (growth inhibition for 24 hours); oxytetracycline had a bactericidal effect at a 0.1 % concentration, and a bacteriostatic effect at 0.01% (growth inhibition for 72 hours).

Ent. faecalis showed the highest sensitivity to antibiotics in this experiment. In essence, the pathogen required minimum concentrations of the tested drugs: 0.001% of pefloxacin, 0.01% of enrofloxacin, and oxytetracycline. The experiment revealed a bacteriostatic effect in 0.0001% and 0.001% concentrations of enrofloxacin and oxytetracycline, respectively. Also, a 0.001% concentration of enrofloxacin inhibited the growth of *Ent. faecalis* for 48 hours, and that of oxytetracycline inhibited the growth only for 24 hours. The test of MPB serial dilutions used to estimate the sensitivity of the pathogens to antibiotics showed that the minimum inhibitory concentration of pefloxacin to all pathogens was 100 mcg/ml (0.01% solution), for enrofloxacin the concentration was 1000 mcg/ml (0.1%solution). In contrast, for oxytetracycline the concentration was 10, 000 mcg/ml (1 % solution). Disc diffusion assays performed for estimating the sensitivity have produced more evident results. Visual evaluation of the grown cultures in the pefloxacin experiment showed that although the plates with the lowest drug concentrations did not result in sterility zones, the growth of the pathogen was prolonged. The observation indicated that pefloxacin produced an inhibitory effect on EFB pathogens even at low concentrations.

At a 0.00001% concentration, slightly distinct zones of inhibition were observed (6-8 mm in diameter) on the plates with *Bac. alvei*. The pathogen tended to increase sensitivity with higher concentrations of the drug gradually; the feature is likely due to the sporulation ability of the pathogen. The 0.1 % concentration of pefloxacin was the threshold for *Bacillus alvei* as higher concentrations resulted in high bacterial sensitivity; the diameter of the sterility zone reached 26 mm.

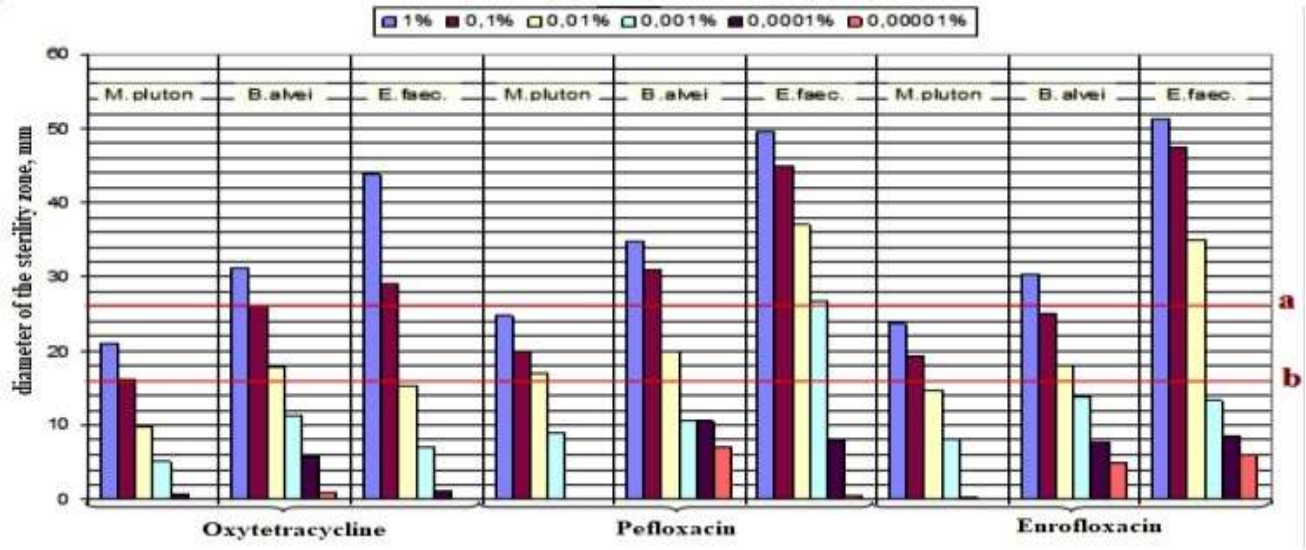

Figure 1: The sensitivity of EFB pathogens to different concentrations of the tested antibiotics. lines a and b describe the boundaries of the sensitivity zones: high sensitivity is above line a, sufficient sensitivity is between lines a and b, low sensitivity is below line b.

However, *Enterococcus faecalis* demonstrated the highest sensitivity to pefloxacin; the bactericidal concentration of the antibiotic appeared as low as 0.001%. A progressive increase in the concentrations produced rapid growth of the pathogen sensitivity, for instance, starting with a 0.01% concentration, the diameter of the inhibition zone was 2-3 times higher than the indices of other pathogens.

Melisococcus plutonium was the most resistant to pefloxacin. At the first two concentrations, cultures of Melisococcus plutonium grew evenly on the entire plate surface and showed low sensitivity at a dose of 0.001% (diameter of the inhibition zone was 7-11 mm). Although *Melisococcus plutonium* and *Bacillus alvei* showed sufficient sensitivity to the drug at the same concentration of 0.01%, *Melisococcus plutonium* stayed in this range even at a 0.1 % concentration while *Bacillus alvei* showed high sensitivity at a 0.1 % concentration. *Melisococcus plutonium* showed high sensitivity to pefloxacin only at a dose of 1%, when the diameter of the inhibition zone was 26 mm.

Visual examination of the grown EFB cultures applied on the enrofloxacin impregnated discs did not reveal inhibition of the colony growth beyond the sterility zone. Enrofloxacin tended to produce uneven, slightly irregular edges of the inhibition zones. The quantitative valuation of the zones involved measuring the diameter of the outer circle (the farthest from the center edges) and the inner circle (the closest to the centre edges) and calculation of the mean value.

Both enrofloxacin and pefloxacin affected *Bacillus alvei* at the minimum experimental concentration of 0.00001%, but the action was weak as the diameter of the sterility zone reached up to 10 mm. The pathogen had a high sensitivity to the drug taken at 1, and 10 % concentrations, the diameter of inhibition zones exceeded 25 mm. At initial concentrations, *Enterococcus faecalis* was a little more resistant to the drug compared to *Bacillus alvei*.

However, a 0.01 % concentration of enrofloxacin was the threshold for *Enterococcus faecalis*, the inhibition zone increased sharply up to 34 mm in diameter, and at a 0.1% concentration, the inhibition zone reached 42-45 mm.

Further increase in the concentration resulted in the sterility zone exceeding half of the entire surface of the seeded lawns (up to 52 mm at 1% concentration and up to 56 mm at a10% concentration), but bacterial growth was not inhibited on the zone edges.

The action of the enrofloxacin initial concentrations on *Melisococcus plutonium* is almost similar to that of pefloxacin. Low sensitivity was detected at 0.001% dose of the drug, and 0.01% concentration resulted in the sterility zone with the diameter exceeding the threshold of 14 mm. The figure indicated sufficient sensitivity of the pathogen to the antibiotic. The gradual growth of the inhibition zone resulted from 0.1% and 1% concentrations of enrofloxacin. *Melisococcus plutonium* showed high sensitivity to enrofloxacin (the diameter of the sterility zone was 27-30 mm) only at a ten %.concentration.

Visual examination of Melisococcus plutonium with the oxytetracycline impregnated disc revealed yellowing of the outer medium around the disc. The change in the colour demonstrated diffusion of the antibiotic from the disc to the medium. Diameter values of the sterility zones formed by oxytetracycline indicated that the antibiotic had approximately an identical effect on pathogens. However, *Enterococcus faecalis* appeared the most sensitive to oxytetracycline, and *Melisococcus plutonium* showed the highest resistance to the drug in the experiment.

Comparative analysis of the effect that the tested antibiotics had on pathogens revealed a higher sensitivity of EFB pathogens to fluoroquinolones compared to oxytetracycline. The disc diffusion assays for estimating the bacterial sensitivity found the ability of enrofloxacin to crystallize and instability of the antibiotic in dense media. The finding suggests that the drug may also appear unstable when added to the bee feed. Therefore, enrofloxacin was rejected for further studies.

The tested drugs were analysed for therapeutic efficacy. The analysis showed that pefloxacin added to the sugar syrup produced the following results: 6 days after treatment, the number of infected larvae in the bee colonies decreased 1.7 fold on average, and 12 days after treatment, the number of the infected larvae fell 16-fold (Figure 2). The spraying of the drug resulted in a 19.6 -fold decrease of the infected larvae 12 days after treatment.

The efficacy was 93.8% and 94.9 % for the two procedures of the drug application. The efficacy of oxytetracycline was comparable with that of pefloxacin six days after the introduction of the drug.

However, the number of dead larvae fell 7.8 fold 12 days after treatment. The efficacy of oxytetracycline was 87.4%.

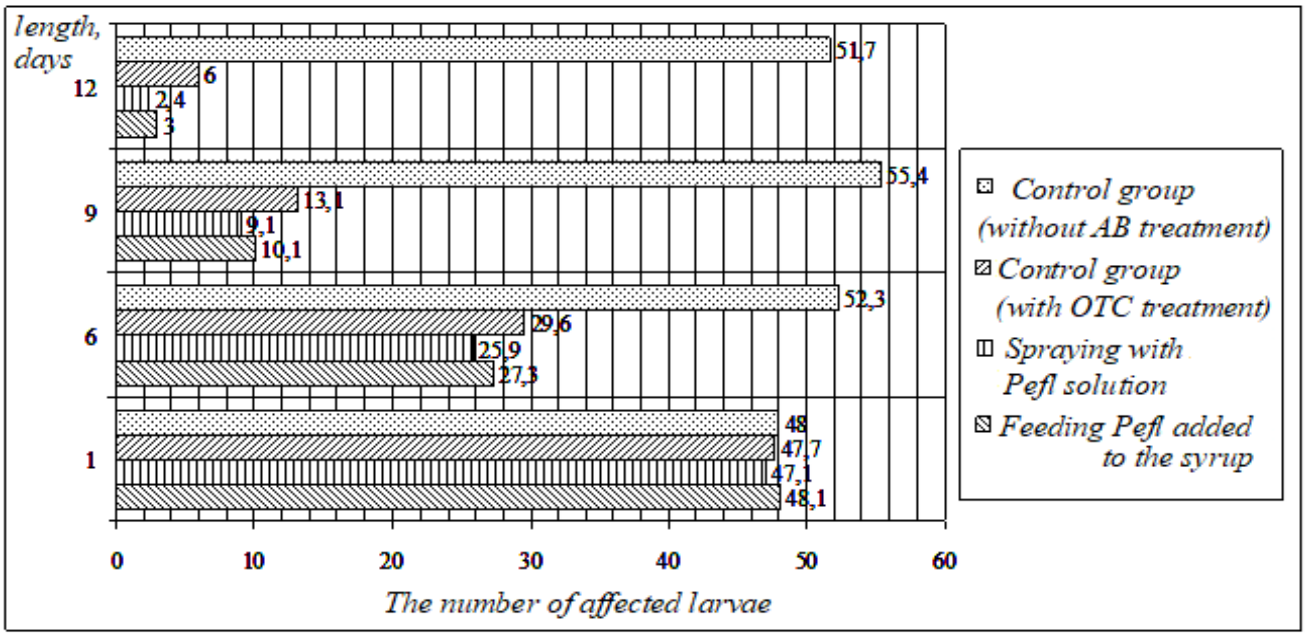

Figure 2: The dynamic pattern of the infected brood after treatment with antibiotics (AB-antibiotic, OTC -oxytetracycline, Pefl - pefloxacin)

The number of infected brood in the control group increased up to day 9 of the experiment. However, by day 12, the number of affected larvae decreased slightly. The tendency was associated with worker bees providing cleaning of the hive.

Discussion

The analysis of the epidemic situation in the Republic of Bashkortostan showed that different districts of the republic had various types of foulbrood disease. European foulbrood disease affects bee colonies in Baymaksky, Kugarchinsky, Nurimanovsky districts, bee colonies are affected by EFB combined with American foulbrood disease in Aurgazinsky and Ufimsky districts, and with ascospherosis in Beloretsky district. The infection takes place secondary to varroosis invasion. Differences are found not only in the secondary microflora.

The primary pathogen *Melisococcus plutonium* was not detected in diseased bee colonies of two districts, although the colonies from where samples were collected showed all symptoms of this disease. The findings suggest that both the virulence and the genetic origin of the host assist the pathological process. The queen bee is responsible for variability in the protective properties of a bee colony to specific pathogens.

To some extent, this suggestion is evidenced by Lewkowski & Erler [6] and McKee et al. [19], who studied the interaction of the host and pathogen, and the impact of *Melisococcus plutonium* on them at the genotype level.

The present study tested pefloxacin and enrofloxacin and compared them with the basic oxytetracycline, commonly used for the purpose. The tested drugs belong to fluoroquinolones. Fluoroquinolones are a group of drugs with marked antibacterial activity against a wide range of microorganisms. Fluoroquinolones inhibit the DNA gyrase and topoisomerase of bacterial cells. As a result, DNA synthesis is disrupted, and bacteria die [20]. Fluoroquinolones have a mechanism of action different from other antibiotics. It reduces the risk of bacterial resistance to fluoroquinolones and the risk of cross-resistance to other antibiotics.

The comparative analysis of the three tested antibiotics included the test of serial dilutions in meat-peptone broth and agar-based disc-diffusion assays. The analysis revealed a higher sensitivity of EFB pathogens to fluoroquinolones than to oxytetracycline. Two secondary invaders, *Enterococcus faecalis* and *Bacillus alvei*, showed high sensitivity to pefloxacin at 0.001% and 0.01% concentrations, respectively.

To oxytetracycline, they showed high sensitivity at 0.1% and 1 % concentrations. *Melisococcus plutonium* demonstrated a little lower sensitivity. Pefloxacin had a significant effect on the pathogen. Sufficient sensitivity to pefloxacin was observed at a 0.01% concentration, to oxytetracycline, sufficient sensitivity was obtained at a 0.1% concentration.

Among the tested drugs, pefloxacin had the lowest concentration (0.01%) required for the complete growth inhibition of the studied pathogens. In contrast, Sanjivan & Rana [20] found that oxytetracycline had the highest inhibitory effect on *Melisococcus plutonium*, while ciprofloxacin (fluoroquinolones) showed a slightly lower index. The difference in the results can assumably be accounted for by different strains of microorganisms used in the experiments.

The study tested drugs for therapeutic efficacy. The experiment showed that pefloxacin was highly effective in treating EFB affected bee colonies. The bee colonies treated with pefloxacin demonstrated the maximum therapeutic effect. The study found that spraying of the diseased combs with the drug was a little more active than introducing the antibiotic to the bee feed. However, the first procedure is more labour-intensive, and the difference in performance indices is unreliable. Therefore, adding the antibiotic to the sugar syrup should be considered the optimal procedure of drug application.

The experimentation showed that oxytetracycline was highly effective. However, the concentration of the active substance in therapeutic solutions was 0.05%. Pefloxacin used almost a five-fold lower concentration. The concentration reduced the risk of contaminating apiary products with antibiotics.

Conclusions

The study has found that in Aurgazinsky, Beloretsky, Kugarchinsky and Ufimsky districts European foulbrood disease is caused by Melisococcus plutonium, Bacillus alvei, and Enterococcus faecalis; in Nurimanovsky district by Bacillus alvei, Enterococcus faecalis and Brevibacillus laterosporus; in Baymaksky district the primary pathogens of the disease are Bacillus alvei and Enterococcus faecalis. In Nurimanovsky and Baymaksky districts, Melisococcus plutonium has not been detected as the primary pathogenic agent. However, Brevibacillus laterosporus has been detected in the apiaries of Nurimanovsky district.

Bee colonies of the studied apiaries in Aurgazinsky, Baymaksky, Beloretsky, Kugarchinsky, Nurimanovsky, and Ufimsky districts (Republic of Bashkortostan) are affected by European foulbrood disease. Aurgazinsky and Ufimsky districts have the infection combined with American foulbrood, and the disease is combined with ascospherosis in Beloretsky district.

The test of serial dilutions in the agar has revealed that the sensitivity rate of EFB cultures to the tested drugs varies in different districts of the republic. Enterococcus faecalis is the most sensitive both to the conventional and candidate antibiotic, and Melisococcus plutonium is the least sensitive. The minimum bactericidal concentration of pefloxacin is 0.001% for Enterococcus faecalis, while the minimum concentration for Brevibacillus laterosporus and Bacillus alvei is 0.01%. Oxytetracycline exceeds the index 10fold, and it is 0.01 % for Enterococcus faecalis and 0.1 % for Brevibacillus laterosporus and Bacillus alvei. The minimum bactericidal concentration of pefloxacin is 0.01% for Melisococcus plutonium, which is 100 times lower than the minimum concentration of oxytetracycline (1.0%).

High therapeutic efficacy is achieved (93.8...95.9%) in treating bee colonies affected by European foulbrood disease with pefloxacin by adding the antibiotic to the feed syrup (1:1) at a dose of 0.01%. The value is significantly higher than the effect of oxytetracycline applied at a dose of 0.05% (87.4...88.2%).

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