A STUDY ON ANTIBACTERIAL ACTIVITY OF RIPHAMPIZYN AGAINST BEE DISEASE MICROORGANISMS

Doç. Dr. Kalinka GURGULOVA, Doç. Dr. Ivan PANCHEV, Doç. Dr. Philip STANCHEV

Doc. Dr Kalinka Gurgulova National Diagnostic Research Veterinary-Medical Institute,
15, P. Slaveykov Blvd., city of Sofia 1606, Republic of Bulgaria
Doc. Dr Ivan Panchev "Primavet – Sofia" Ltd., 275, Slivnitsa Blvd., P.O.Box 9, city of Sofia 1528, Republic of Bulgaria
Doc. Dr Philip Stanchev "Primavet – Sofia" Ltd., 275, Slivnitsa Blvd., P.O.Box 9, city of Sofia 1528, Republic of Bulgaria

INTRODUCTION

Bee bacterial diseases present a problem in bee pathology. Different methods of healing such diseases have been presented.

Rifampicine is used in veterinary medicine, as well as in apiculture. The agents of putrefaction diseases in bee nest are sensitive to this antibiotic.

There have been examined the possibilities for development of an optimized method of applying rifampicine in the bee family via specific preparation Biopapir ® -strips.

MATERIAL AND METHODS

25 Paenibacillus larvae strains and 10 Bacillus alvei strains have been used in the experiment on sensitivity tracing. The strains are characteristic of the types indicated. For the purpose of their cultivation it has been used a solid Muler – Hinton medium with 10 % horse serum. The strains sensitivity has been tested according to the routine methods of determining the minimum lowering concentrations, approved by the World Health Organization, as compared to the corresponding dilutions of tetracycline, tilosine, streptomycin and rifampicine.

The clinical experiments were carried out within the period of 1999-2000 on 150 bee colonies in a private apiary in the town of Svishtov (10 colonies with clinically manifested AF), 50 bee colonies in a private apiary in Sofia area (2 colonies with clinically manifested AF) and 53 bee colonies from the experimental apiary of AF at TU–Stara Zagora (3 colonies with clinically manifested AF). The colonies had a development normal for the season. We liquidated the clinically sick colonies and after that created experimental groups (130, 38 and 40 colonies, respectively) and 1 control group of 10 colonies in each area. We applied two times to the experimental colonies the Biopapir dose indicated by the producer – 2 bands, each of them containing 100 mg of Rifampicin, two times at an interval of 7 days. We applied the prevention treatment in three seasons (autumn of 1999, spring of 2000 and autumn of 2000). The control colonies were left untreated for 1 month after the disease manifestation in the autumn of 1999. After that we applied Biopapir according to the same scheme.

During the whole experimental period we monitored the treated and control colonies for normal development, manifestation of side phenomena and harmful effect of the tested preparations on the queen, bees and brood, normal overwintering and manifestation of relapses.

On the 30^{th} and 40^{th} day after the first treatment (on the 20^{th} and 30^{th} day after the second treatment, respectively) we took honey samples in order to determine the Rifampicin level. We used a microbiological diffusion method with strain of *Bacillus subtilis* 6633 ATCC on agar medium, at a control with a standard containing the corresponding Rifampicin concentrations.

PURPOSE

1. Tracing Paenibacillus larvae strains and Bac.alvei strains sensitivity to rifampicine and other antibiotics /mg/ml/

2. Clinical test of Biopapir® - strips

RESULTS AND DISCUSSION

The results from the prevention treatment are reported in Table 2.

In all three apiaries, we found a 100% effect from the two-band double prevention treatment during the three seasons – the autumn of 1999, the spring of 2000 and the autumn of 2000. No relapses were found. In the control groups (Table 2), clinical manifestations of AF were found in 3 colonies from the Svishtov apiary, in 2 colonies from the Sofia apiary and in 2 colonies from the Stara Zagora apiary. After the Biopapir treatment, a 100% effect was found for them and no relapses were observed, as it was for the control groups. This shows that it is necessary to do a prevention treatment with chemotherapeutics of the colonies without clinical manifestations from bad apiaries. Otherwise, the disease manifests itself in the untreated colonies and the beekeepers suffer losses from their liquidation. The preparation Biopapir also may be used for treatment of European foulbrood, parafoulbrood and bacterial diseases among adult bees owing to the high antibacterial activity of Rifampicin to the causing agents of these diseases, the easy application and the precise dosage.

The results we obtained confirm the investigations of the Russian researchers for the application of Bactopol (Grobov et al., 1997; Sotnikov et al., 1997).

CONCLUSION

The results obtained from the clinical test of Biopapir show a 100% effect for prevention of American foulbrood by double application at an interval of 7 days. The dose is determined by the bee colony strength. 2 bands at an interval of 7 days are recommended for strong colonies with normal development. 1 band at an interval of 7 day as applied for splits and weaker colonies. No residual amounts in the honey have been detected after 30 days from the treatment start – the putting of the bands. Since part of the bands is thrown out if placed vertically between the frames, we recommend they to be placed on the top of the frames.

The advantages of Biopapir consist in the following:

- High antibacterial activity towards the causative agents of bacterial diseases of the bee colony;
- Precise dosage of the preparation;
- Safe methods of absorption by bees and larvae. The cleaning bees gnaw the bands and absorb Rifampicin. When they become nurse bees, they feed it to the brood;
- Easy application;
- Economical treatment since no sugar is used;
- Harmless for the bees and brood;
- No danger to enter the honey for sale;
- Non-toxic for people.

Species microorganism	Tetracyclin	Tylosin	Strepto-mycin	Rifampicin
Paenibacillus larvae	16	4 – 16	32	0,06 – 0,25
Bac. alvei	8 – 16	4 – 8	16 –32	0,06 – 0,12

Level of sensibility species Paenibacillus larvae and species Bac. Alvei to Rifampicin and others anti-infectious agents /μg/ml/ Table 1.

The rates of the minimum lowering concentrations (table 1), have their lowest values with rifampicine, and their highest values with streptomycin. The results obtained confirm the opinion, expressed by a number of scientists, that the agents of bee bacterial diseases possess higher degree of resistance against tetracycline antibiotics and streptomycin. The rifampicine results show that it has good perspectives for treatment of bee bacterial diseases.

Table 2. Effect for prevention of American foulbrood by Biopapir

Town	Group	Number bee family	Treatment	Recidiv		
				1999	2000	2001
Svishtov	Experimental	100	2 strips	-	-	-
	Control	10	- *	3	-	-
Sofia	Experimental	38	2 strips	-	-	-
	Control	10	- *	2	-	-
Stara Sagora	Experimental	40	2 strips	-	-	-
	Control	10	- *	2	-	-

∗ Treatment one month after experimental bee family in the autumn 1999

KAYNAKLAR

Gurgulova, K., ve ark. 1988. Bazı bakteri suşlarının antimikrobiyal etkinliğe karşı karşılaştırmalı direnci. Trut. Derg. IV. Genç Bilim Adamları Sempozyumu 27-28 Ekim Kazanlık, Bulgaristan.

Korudjiski, N., ve ark. 1980. Dondurulmuş boğa sperminden izole edilen Pseudomonas aeruginosa suşlarına karşı antibiyotik (sulfonamid, furozolidon) duyarlılığı. Ved. Med. J. No: 9-10, 3-7.

Korudjiski, N., ve ark. 1985. Endometritli sığırlardan izole edilmiş proteus mirabilis suşlarının virulensi ve ilaçlara direnci. IV. Mikrobiyoloji Kongresi 13-15 Ekim. Varna, Bulgaristan.

Sotnikov, A., 1997. Yavru çürüklüğüne karşı yeni bir ilaç. Arıcılık Derg., Baktopol, Rusya.

Shimanuki. H., 1998. Oxytetracycline karşı Paenibacillus larvae suşlarının direnci. Apimondia 1999. Vankovar, Kanada.