THE INFLUENCE OF THE CHOSEN CHEMOTHERAPEUTICS ON THE INTESTINAL FLORA OF THE HONEYBEE

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Introduction

The examinations of microflora in the honeybee *Apis mellifera* L., began in the 19th century and they concerned mainly the identification of pathogenic bacteria. Later, many authors tried to determine the microflora of the honeybee alimentary tract, but the results of studies on the occurrence of intestinal flora in workers differ significantly. Some authors such as White (1921), Lotmar (1964), Trilenko (1965) and Gilliam (1971) state that the intestinal content of normal larvae, pupae and freshly emerged bees is sterile. Poltev *et. al.* (1969) present another view, stating that honeybee larvae are free of bacteria until they are 3 days old, and that only between the third and fifth day of life microorganisms are present in their midguts.

The aim of this research is:

1. Determination of the numbers and kinds of bacterial flora in the midgut of young honeybees (0—24 hours old).

2. Examination of how some chemotherapeutics used in honeybee colonies such as Penicillin, Streptomycin, Polisulfamid, Ascocidin, Fumagillin and TCL can influence the bacterial flora of the honeybee worker midgut.

To date, these problems have been treated only in a fragmentary form making the evaluation of the influence of chemotherapeutics applied in honeybee therapy on bacterial flora of the alimentary tract in healthy bees quite impossible.

Material and Methods

These studies were performed on 1000 very young workers from disease free colonies in the Koszalin and Warsaw provinces.

The samples were taken from:

— very young bees collected from honeycombs just after their emergence;

— bees (0—24 hours old) fed therapeutic doses of the examined chemotherapeutics for 24 hours. Control bees were fed sugar syrup for the same time.

The workers were decapitated and their midguts were isolated. The homogenates obtained from each midgut were inoculated onto a nutrient agar with 5.0% sheep blood. The inoculated agar media was incubated aerobically at 37°C until a good bacterial growth was noted. Single colonies from the

culture were then inoculated on Chapman and Levin agar media. The midgut contents of the worker bees treated with chemotherapeutics were inoculated into a nutrient agar with 5.0% sheep blood and also into Mac Conkey agar medium.

The isolates were identified on the basis of morphological, cultural and biochemical properties according to Bergey's taxonomy. Bacteria from the genus *Enterobacteriaceae* were determined by the API system. Moreover, movement, oxidative and fermentative properties and the character of growth on Mac Conkey agar were examined. The reference strains were obtained from the Laboratory of Salmonellae Bacteriology Institute of the State Hygiene Institute in Warsaw, from the Apicultural Institute in Dol and from the Czechoslovakia Agricultural Academy.

The following chemotherapeutics were used: Fumagillin DCH, Ascocidin, TCL, polisulfamid, Penicillin and Streptomycin.

The sensitivity of the bacterial strains was determined by a routine disc method. *Staphylococcus aureus* 209P with a known sensitivity to antibacterial drugs served as a control.

The numbers and kinds of bacteria in the midgut of workers fed therapeutic doses of chemotherapeutics was determined on bees kept in cages at 37°C, 98% RH in groups of 20 individuals each. Before feeding medication, insects were starved for 9 hours. The treatment bees were then individually fed medicated syrup for 24 hours. Controls kept under the same conditions were fed a pure syrup (no medication) for 24 hours. The bees were then transferred into a sterile box and their midguts were isolated. The numbers and kinds of bacteria as well as their frequency in the alimentary tract was determined as above. All determinations were carried out three times and the results were statistically analyzed by the method of Gmurman and Greń.

Results

The studies performed on 160 very young worker bees revealed that the alimentary tract contained bacterial flora of the genera *Bacillus, Enterobacter, Escherichia, Micrococcus, Staphylococcus* and *Streptococcus.* The composition of the bacterial flora changed according to the season of the year (Table 1, 2). *Enterococcus* and *Micrococcus* sp. dominated in spring, however, *Bacillus* sp. dominated in summer. One of the most harmful bacterial diseases caused by *Bacillus* larvae, American foulbrood develops as a rule in summer when the growth of *Bacillus* sp. is luxurient. However, European foulbrood, with an equally wide range of occurrence as American foulbrood, appears in spring. Its appearance is strictly connected with the cold season and a lack of sufficient food. Frequency of occurrence of bacteria was different in each period of the year. (Table 3).

Table 1

Bacteria isolated from midgut of very young bees *(Apis mellifera)* studied in July 1982

Bacteria	Number of strains	Percentage
Bacillus	31	59.61
Enterobacter	4	7.69
Escherichia	2	3.85
Micrococcus	3	5.77
Staphylococcus	3	5.77
Streptococcus	9	17.31
Total	52	100.00

Table 2

Bacteria isolated from midgut of very young bees *(Apis mellifera)* studied in late April and early May 1983

Bacteria	Number of strains	Percentage
Bacillus	9	15.79
Enterobacter	23	40.35
Escherichia	4	7.02
Micrococcus	16	28.07
Streptococcus	5	8.77
Total	57	100.00

Table 3

Seasonal sample size of bees *(Apis mellifera)* studied, number and percentage of midguts free from bacteria

Season	Number of bees studied	Number of midguts free from bacteria	Percentage
Spring	80	28	35.00
Summer	80	35	43.75
Total	160	63	39.37

In the studies presented here, the contents of alimentary tracts in 35% of the workers examined in spring were sterile. In summer, bacterial flora was not isolated from 43.75% of worker bees. Therefore, more than 9.0% of the individuals in summer were free of bacterial flora in the alimentary tract. This is probably the result of increased royal jelly ingestion at that time. In summer, strong colonies can have a few nurse bees for one larva whereas in spring, when colonies are weaker the number of nurse bees is lower. Therefore, one can suspect that spring brood does not use much royal jelly, but subsequent young bees can easily feed on royal jelly which sterilizes their

alimentary tract.

Results of determinations of the intestinal flora, their sensitivity to some chemotherapeutics and the influence of these chemotherapeutics on the bacteria in the alimentary tract are given in Table 4 and 5.

All the isolates both from very young bees (Table 4) and from workers fed chemotherapeutics (Table 5) were sensitive to Penicillin and Streptomycin and their sensitivity to Polisulfamid varied. The isolates were resistant to TCL, Ascocidin and Fumagillin DCH. The administration of these chemotherapeutics in therapeutic doses influenced the kind of bacteria isolated and moreover, changed the total number of microorganisms present. Fumagillin DCH (Table 6) increased the number of *E. coli* and reduced the number of *Micrococci.* It seems that some components present in Fumagillin DCH create better conditions for the development of *E. coli.* Observations on the effectiveness of Ascocidin (Table 7) revealed that this polyene antibiotic which is active against *Ascosphaera apis* is devoid of antibacterial activity. Therefore, in treated colonies, the saprophytic bacterial flora of the intestines is stable.

TCL (Table 8) did not exert any influence on bacterial flora of the intestines in very young bees. TCL added to sugar syrup reduced the number of *Escherichia coli, Enterobacter* sp. and *Bacillus* sp. It would be interesting to note in further studies the character of these changes and their consequences for the bee colony. Polisulfamid (Table 9) eliminated *Streptococcus* sp., *Staphylococcus* sp. and *Micrococcus* sp. from the midgut, as well as reduced considerably the number of *E. coli* and *Bacillus* sp. Penicillin (Table 10) influenced considerably the number of *E. coli, Enterobacter* sp., *Streptococci, Staphylococci* and *Micrococci.* Streptomycin (Table 11) completely eliminated *Micrococci* and reduced the number of *E. coli, Enterobacter* sp., Bacillus sp. and *Staphylococci.*

Statistical analysis of the changes in the total number of bacteria and the proportion of those in the *Enterobacteriaceae* family after the application of chemotherapeutics as compared to controls showed that there is no statistically significant influence of Fumagillin DCH and Ascocidin on the total number of bacteria found.

Taking into account a considerable reduction in the number of bacteria by chemotherapeutics (especially by Penicillin, Streptomycin or Polisulfamid, which are commonly used for the control of honeybee diseases as stimulators of colonies) the danger of developing some unfavorable bacteria or yeast in the alimentary tract of treated workers is real.

Conclusions

1. In a considerable percent of very young worker bees under hive conditions, the intestinal tract is free of *Escherichia coli, Enterobac-*

Table 4

Sensitivity of bacterial strains isolated from the midguts of very young bees *(Apis mellifera)* to chemotherapeutics

Kind of microorg.	Number of strains	Fumagillin			Ascocidin			TCL			Polisulfamid			Penicillin			Streptomycin		
		+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+
Escherichia	6	0	0	0	0	0	0	0	0	0	0	3	3	0	2	4	5	1	0
Enterobacter	27	0	0	0	0	0	0	0	0	0	4	5	18	1	3	23	14	11	2
Strepto-coccus	14	0	0	0	0	0	0	0	0	0	7	4	3	11	3	0	10	4	0
Bacillus	40	0	0	0	0	0	0	0	0	0	31	5	4	38	2	0	33	5	2
Staphylo-coccus	3	0	0	0	0	0	0	0	0	0	2	1	0	2	1	0	3	0	0
Micrococcus	19	0	0	0	0	0	1	0	0	0	12	5	2	16	3	0	8	6	5
Total	209																		

+++ sensitive, ++ less sensitive, + slightly sensitive, 0 resistant

Table 5

Sensitivity of bacterial strains isolated from the midguts of bees fed therapeutic doses of chemotherapeutics

Kind of microorg.	Number of strains	Fumagillin			Ascocidin			TCL			Polisulfamid			Penicillin			Streptomycin		
		+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+
Escherichia	36	0	0	0	0	0	0	0	0	0	0	0	7	0	0	3	28	5	3
Enterobacter	18	0	0	0	0	0	0	0	0	0	0	0	9	11	7	0	15	3	0
Strepto-coccus	11	0	0	0	0	0	0	0	0	0	0	0	5	4	2	3	3	5	0
Bacillus	32	0	0	0	0	0	0	0	0	0	0	4	12	25	6	1	27	3	2
Staphylo-coccus	7	0	0	0	0	0	0	0	0	0	0	1	3	6	1	0	7	0	0
Micrococcus	16	0	0	0	0	0	0	0	0	0	0	3	8	11	5	0	8	5	3
Total	120																		

+++ sensitive, ++ less sensitive, + slightly sensitive, 0 resistant

Table 6

Number of strains and percentage of each kind of bacteria found in midgut of *Apis mellifera* with application of Fumagillin in treatment (n=60) and control (n=60) groups

Kind of microorganisms	Fumagillin (treatment)		Control (no. treatment)	
	Number of isolates	%	Number of isolates	%
Escherichia	22	68.76	16	41.03
Enterobacter	4	12.50	4	10.25
Streptococcus	3	9.37	2	5.13
Bacillus	2	6.25	13	33.33
Staphylococcus	1	3.12	2	5.13
Micrococcus	0	0	2	5.13
Total	32	100.00	39	100.00

The microorganisms were found in 27 (45%) bees from the treatment group and in 27 (45%) bees from the control group.

Table 7

Number of strains and percentage of each kind of bacteria in midgut of *Apis mellifera* with application of Ascocidin in treatment (n=60) and control (n=60) groups

Bacteria	Ascocidin (treatment)		Control (no treatment)	
	Number of isolates	%	Number of isolates	%
Escherichia	14	40.00	17	36.96
Enterobacter	4	11.43	7	15.21
Streptococcus	3	8.57	5	10.87
Bacillus	8	22.86	12	26.09
Staphylococcus	2	5.71	2	4.35
Micrococcus	4	11.43	3	6.52
Total	35	100.00	46	100.00

The microorganisms were found in 25 (41.67%) bees from the treatment group and in 32 (53.33%) bees from the control group.

Table 8

Number of strains and percentage of each kind of bacteria in midgut of *Apis mellifera* with application of TCL in treatment (n=60) and control (n=60) groups

Bacteria	TCL (treatment)		Control (no treatment)	
	Number of isolates	%	Number of isolates	%
Escherichia	12	38.71	15	35.72
Enterobacter	1	3.23	3	7.14
Streptococcus	2	6.45	3	7.14
Bacillus	7	22.58	12	28.57
Staphylococcus	2	6.45	3	7.14
Micrococcus	7	22.58	6	14.29
Total	31	100.00	42	100.00

The microorganisms were found in 25 (41.67%) bees from the treatment group and in 23 (38.33%) bees from the control group.

Table 9

Number of strains and percentage of each kind of bacteria in *Apis mellifera* with midgut application of Polisulfamid in treatment (n=60) and control (n=60) groups

Bacteria	Polisulfamid (treatment)		Control (no treatment)	
	Number of isolates	%	Number of isolates	%
Escherichia	7	53.84	14	35.90
Enterobacter	3	23.08	2	5.13
Streptococcus	—	—	3	7.69
Bacillus	3	23.08	12	30.77
Staphylococcus	—	—	2	5.13
Micrococcus	—	—	6	15.38
Total	13	100.00	39	100.00

The microorganisms were found in 11 (18.33%) bees from the treatment group and in 26 (43.33%) bees from the control group.

Table 10

Number of strains and percentage of each kind of bacteria in *Apis mellifera* with midgut application of Penicillin in treatment (n=60) and control (n=60) groups

Bacteria	Penicillin (treatment)		Control (no treatment)	
	Number of isolates	%	Number of isolates	%
Escherichia	19	45.25	26	45.61
Enterobacter	3	7.14	5	8.78
Streptococcus	2	4.76	3	5.26
Bacillus	12	28.57	12	21.05
Staphylococcus	1	2.38	3	5.26
Micrococcus	5	11.90	8	14.04
Total	42	100.00	57	100.00

The microorganisms were found in 30 (50%) bees from the treatment group and in 37 (61.67%) bees from the control group.

Table 11

Number of strains and percentage of each kind of bacteria in *Apis mellifera* with midgut application of Streptomycin in treatment (n=60) and control (n=60) groups.

Bacteria	Streptomicin (treatment)		Control (no treatment)	
	Number of isolates	%	Number of isolates	%
Escherichia	13	48.16	23	36.51
Enterobacter	3	11.11	6	9.52
Streptococcus	1	3.70	5	7.94
Bacillus	9	33.33	15	23.81
Staphylococcus	1	3.70	5	7.94
Micrococcus	—	—	9	14.28
Total	27	100..00	63	100.00

The microorganisms were found in 24 (40%) bees from the treatment group and in 34 (56.67%) bees from the control group.

ter, Streptococcus, Bacillus, Staphylococcus and *Micrococcus.*

2. The kind of bacterial flora of the midgut in very young workers varies seasonally (spring and summer). In spring, *Enterobacter* sp. and *Micrococcus* sp. dominate, but in summer *Bacillus* sp. and *Streptococcus* sp. are dominant.

3. Penicillin, Streptomycin and Polisulfamid used in therapeutic doses in sugar syrup for 24 hours caused a statistically significant decrease in the number of bacteria in midguts of worker bees. Fumagillin DCH increased the number of *Escherichia* sp., but TCL reduced the number of *Enterobacter* and *Bacillus* sp.

4. Ascocidin is devoid of any antibacterial activity.

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