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TRANSMISSION OF *PAENIBACILLUS LARVAE SPORES* THROUGH SOCIAL CONTACTS WITHIN HONEYBEE COLONIES (APIS MELLIFERA CARNICA POLLMAN, 1879)[4]

ABSTRACT

American foulbrood is a widely distributed, very persistent honeybee disease, caused by the spore forming bacterium Paenibacillus *larvae. The disease is highly contagious and unless recognized at an early phase, can give rise to significant losses for beekeepers. The purpose of this study was to investigate what would happen with the level of P. larvae spores concentration in honey sacs during the transmission of food through social contacts and during various times of digestion of the spore contaminated food. A method described by Ritter and Kiefer (1993) for determining the presence and the number of P. larvae spores in honey samples was modified in a way that through the number of the grown P. larvae colonies on MYP agar it was possible to determine the concentration of P. larvae spores in the honey sac of individual bees. Regardless of the quantity of food delivered through social contacts, the known concentration of P. larvae spores remained unchanged. After various times of digestion, the concentration of P. larvae spores was also constant. Therefore, the ability of honeybees to reduce the number of P. larvae spores from their honey sac by filtration was not proven.*

Key words: Paenibacillus *larvae, spores, filtration, honey sac*

1. INTRODUCTION

American foulbrood (AFB) is a widely distributed and very persistent disease of the larval honeybees, caused by the spore forming bacterium *Paenibacillus larvae* (Genersch et al., 2006, former *Bacillus larvae* (White, 1906)). The disease is usually not recognised until signs of the infection are detected in hives during routine hive management procedures, and often by the time the symptoms have been spotted, it is too late to save the colony (DiemerI, 1988).

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P. larvae spores remain viable almost indefinitely on bee keeping equipment and on hives (Shimanuki, 1990). The number of spores able to trigger AFB can be highly variable because factors, such as the environment and the hereditary resistance of the bees to the disease, are also playing a part in it (Hansen et al., 1988; 1989).

In this work, the epidemiology of AFB was investigated from the aspect of distribution of spores through social contacts of honeybees, similar to those occurring within the colony during regular life activities of bees. The purpose was to find out which quantities of honey single bees deliver through social contacts and whether repeated social contacts influence the level of concentration of *P. larvae* spores in honey sacs. The aim was also to see which quantity of the content of honey sac bees digest during various periods of time and if the concentration of *P. larvae* spores in the honey sac changed during various times of digestion. It was also investigated whether different concentration levels of *P. larvae* spores had any influence on the above mentioned points.

The examinations of honey samples from Germany (Ritter, 1990; 1992), from Australia (Hornitzky, Clark, 1991) and from Norway (Hagen, Hetland, 1988) have shown that contaminated honey is more widespread than expected from the actual number of apiaries with AFB diseased colonies. As AFB can be spread by the honey of the infested bee colonies (Lindström et al., 2008), various methods have been provided for the detection of *P. larvae* spores in honey (Bailey, Lee, 1962; Hansen 1984a; Lloyd, 1986; Shimanuki, Knox, 1988; Sturtevant, 1932; 1936). The most convenient method to diagnose *P. larvae* spores in honey samples has proven to be one described by Ritter and Kiefer (1993). It was developed from the earlier Danish investigations of honey samples (Hansen 1984a; 1984b; Hansen, Rasmussen, 1986). By using Ritter and Kiefer (1993) method it is possible to detect low quantities of *P. larvae* spores in honey (119 +/- 15 spores per gram) and it can be employed in all laboratories without any problem.

2. MATERIALS AND METHODS

2.1 Determination of *P. larvae* spores in honey

From each honey sample, 3 times 5 grams of honey was put in 3 beakers together with 5 milliliters of demineralized water. The suspensions were heated at 90^{O} C, for 5 minutes, in order to kill other unwanted microorganisms. From each beaker 80 microliters were placed on 3 Petri dishes containing Müller - Hinton - Broth - Yeast extract - Natriumpyruvat - Agar (MYP Agar) done after Dingman and Stahly (1983) and according to practical instructions from Dr. Ritter (personal communication), and were placed at 36^{O} C. As MYP - agar is not selective for *P. larvae,* to distinguish the grown colonies the best way was to use the Catalase - Test (Cowan, Steel, 1974; Hayens,1972). Catalase is present in most plant and animal cells. It causes hydrogen peroxide to decompose into carbon dioxide and water. To perform the test, a few drops of 10 percent hydrogen peroxide were superimposed upon young bacterial growth on agar. An immediate vigorous evolution of gas bubbles signified a positive response, and if gas bubbles were not seen, the result was negative (*P. larvae* is catalase negative) Catalase positive strains of *P. larvae* are unknown and most other aerobic sporeforming bacteria are catalase positive (Gordon et al., 1973)**.**

In the case that the Catalase Test shows a positive reaction and *P. larvae* colonies do not develop (Ritte, Kiefer,1993), a bacteriological cultivation method on Columbia - Blood - Slant - Agar was used to finally exclude the *P. larvae* presence in examined material. *P. larvae* could be identified within three to four days due to the formation of giant whips in the fluid part of the culture (Plagemann, 1985), which could be seen (after mixing it with a drop of nigrosin) by microscopy magnification: 1000 x).

2.2 Determination of the number of *P. larvae* spores in honey sacs

The method described by Ritter and Kiefer (1993) for examining the samples of honey was modified, because in dealing with honey sacs, the quantities were much smaller. The method was modified in a way that the final reading of the results was the same, regardless of the examined quantity of honey. For that purpose, special little test tubes were used, and for each honey sac, according to its weight, the quantity of distilled water which had to be added was mathematically worked out. In cases when it was impossible, because the content of the honey sac was not big enough to be diluted and to fill 80 microliter of suspension in the micropipette, honey sacs of 2 to 3 bees from the same group were put together. Because of the low quantity of the content of the honey sacs, in most cases, just one Petri dish was used for one honey sac. After the material was inoculated in Petri dishes containing MYP - agar, they were incubated at 37° C for three days, and later the results were confirmed using the Catalase test, which proved to be a simple, fast and reliable test for excluding all catalase positive bacteria and to confirm the presence of *P. larvae* colonies.

2.3 Preparation of the known concentration of *P. larvae* spores from the infected material

The known concentration of 10^6 *P. larvae* spores per 1 milliliter was prepared from a heavily infected material. The infected bodies of the honey bee larvae were first mixed with sterile demineralized water and the suspension of *P. larvae* spores was then filtrated through the cotton material. The number of spores was determined by counting them in a Neubauer blood count chamber. The computation was carried out following Drews (1976). As the number of spores was initially too high to be counted, the suspension was diluted (1 : 15).

Once the concentration of *P. larvae* spores was known it was easy to prepare the desired concentration by adding the required volume of water. The desired concentration was needed to simplify further calculations, when a number of diluted solutions were made from the spore suspension.

2.4 Preparation of a sugar solution with known concentration of *P. larvae* spores

A sugar solution with a known concentration of *P. larvae* spores was prepared in a way that the number of *P. larvae* colonies which was desirable to grow was 50 colonies in one Petri dish.

In the original paper, Ritter and Kiefer (1993) mixed 5 grams of infected honey with 5 milliliters of demineralized water, and after the treatment in a water bath at 90° C, they placed 80 microliters of that honey solution in the Petri dishes. Each grown colony represented 119 ± 15 spores per gram of honey, but as the honey sacs contained much less than 5 grams, it was

necessary to dilute the content of the honey sacs with water (in the proportion of 1 milligram : 1 microliter).

The total number of *P. larvae* spores in 5 grams of honey, needed to provide the desired growth of 50 colonies in one Petri dish was:

N_{sp} = 50 [colonies] x 119 [spores/gram] x 5 [gram] = 29750 spores

(N_{sp} - number of *P. larvae* spores)

It was also necessary to determine the volume of 5 grams of honey (1.4 gram/milliliter was taken as the specific density of honey):

$V_{honey} = m_{honey} / d_{honey}$ = 5 [gram] / 1.4 [gram/milliliter] = 3.57 [milliliters]

(d_{honey} - density of honey [gram/milliliter]; m_{honey} - mass of honey [gram];

V_{honey} -volume of honey [milliliter])

From the above two results, it was possible to determine the concentration of *P. larvae* spores in 1 milliliter of honey:

$C_{P. larvae\ spores} = N_{sp} / V_h$ = 29750 [spores] / 3.57 [milliliter] = 8333 [spores/milliliter]

($C_{P. larvae\ spores}$ - concentration of spores in solution [number of spores/milliliter])

That information was essential for making a sugar solution of the same concentration 8.333 x 10^3 [spores/milliliter]. The obtained sugar solution was then used as a basic one, and all other dilutions used in the experiments were made out of it.

It was mathematically calculated which volume of *P. larvae* suspension with 10^6 *P. larvae* spores per one milliliter should be mixed with sugar solution in order to obtain such a concentration of *P. larvae* spores, that would enable the growth of 50 *P. larvae* colonies on MYP - agar in Petri dishes, as described before.

Ten milliliters of the basic sugar solution with the known concentration of *P. larvae* spores was enough to perform all the experiments:

N_{sp} = 8.333 x 10^3 [spores/milliliter] x 10 [milliliter] = 8.333 x 10^4 spores

$V_{SpS} = N_{sp} / C_{SpS}$ = 8.333 x 10^4 [spores] / 10^6 [spores/milliliter] = 1.134 x 10^{-3} milliliters

(V_{SpS} - volume of the spore suspension with 10^6 *P. larvae* spores/milliliter;

C_{SpS} - concentration of the spore suspension)

In order to obtain 10 milliliters of a sugar solution with the known concentration of *P. larvae* spores, 1.134 microliters of the spore suspension was put in the beaker, which was then filled up to the level of 10 milliliters with the sugar solution with specific density similar to the one in honey.

Once such a basic sugar solution with the known concentration of *P. larvae* spores was ready, various dilutions of *P. larvae* concentration were made by adding more of the sterile sugar solution.

2.5 Feeding bees with prepared sugar solution and measuring the weight of their honey sac

Before the process of feeding began, bees were isolated and left without food for 6 to 10 hours (it depended on the quantity of honey which bees already had in their honey sacs). In most cases, after 6 hours, their honey sacs were almost completely empty, and after 10 hours there was no food in their honey sacs.

For individual isolation, the best solution were small PVC - test tubes, with a capping on the top. The bottom side of the test tube was cut off in a way that the hole was big enough for a single honey bee to put its head out of it, but too small to escape. In this manner bees were placed close to the food and as they were hungry, they immediately started taking it. They were fed *ad libitum*, and then used in the experiments.

When the bees needed to be isolated as a group, ordinary queen cages made of PVC were used in a way that the desired number of bees was easily and quickly placed in a certain cage. The holes of the cages were just big enough for the bees to use their tongues to reach the food.

The content of the honey sacs was measured either in a way that first the weight of the hungry bee was measured together with the container, and then it was repeated after the bee was fed. The obtained difference (in milligrams) signified the content of the honey sac. When that was not possible, honeybees were first killed, and then the weight of their honey sacs was measured directly.

2.6 Determination of honey consumption level in honey sacs

Two experiments were carried out to control the consumption of honey in honey sacs of bees:

In the first one 60 honeybees were isolated, and after starvation fed *ad libitum*. The weight of each honeybee was measured before and after the feeding, so that the obtained difference signified the food intake. As the honey sacs were empty, the food intake was taken as initial weight of the honey sacs. In each of the six groups of bees, ten single honeybees were individually isolated in small PVC test tubes, as previousely described.

The first group of 10 honeybees was killed (by hypothermia) 10 minutes after they were fed, the second group was killed 20 minutes after they were fed, and other groups were killed 30 minutes, 60 minutes, 90 minutes and 120 minutes after the food intake.

In that way it was possible to compare the level of honey consumption for each group.

Within the second experiment, honeybees were isolated in queen excluders, and they were also fed after the starvation period. Sixty honeybees were isolated in 12 queen excluders, each containing 5 bees. Half of the bees were killed after 6 hours and the other half 18 hours after they were fed. From each group of five bees, their honey sacs were measured, but as starvation periods were long, and the individual contents of honey sacs were sometimes very low, at the end only their average weight was taken into calculation. Eighteen hours after the bees were fed *ad libitum*, the honey sacs were in most cases completely empty.

2.7 Determination of the quantity of honey transferred through social contact

In order to determine the quantity of honey which one hungry bee would receive from another bee which had just been fed *ad libitum*, five hungry bees were put close to the fed ones in a way that they could feel and touch each other only by tongue.

After they were put in such a position, a food transfer started almost immediately. The weight of both bees was measured before one of them was fed *ad libitum* and also soon after the food transfer was over. To control the results, both bees were killed, and their honey sacs were also weighted.

In further experiments, social contacts with one fed bee and two, three, four and five hungry honey bees were examined in a way that for each group, five parallel examinations were done. When the (marked) fed bee was put in the queen excluding cage with the hungry ones, they gathered around her, and the social delivery of the food started immediately. Once the social contacts were over, the weight of all honey sacs was measured.

2.8 Method for determining the transmission of *P. larvae* spores through social contact

To determine the level of transmission of *P. larvae* spores through social contacts, honeybees were first isolated, and after starvation, five groups of five bees (in each group) were fed *ad libitum* with the sugar solution containing the known concentration of *P. larvae* spores. The sugar solution was made in a way that it enabled the growth of approximately 50 colonies of *P. larvae* on MYP - agar in Petri dishes.

In order to examine if the level of the concentration of *P. larvae* spores was influenced by the number of food transfers, each fed bee was first let near one hungry bee, until it gave the hungry bee some food. Then the first bee was killed, and the other one was left to the second hungry bee and at the end it was all repeated with the third hungry bee, so that each time a lower quantity of food was delivered through social contacts. The experiment was repeated five times. Each time 5 bees were fed *ad libitum* and 15 hungry ones were fed through social contacts. All together, 100 honey bees were individually isolated and used for the examinations.

Immediately after social contacts were over, the bees were killed, and their honey sacs were weighed and placed in small test tubes. The contents of their honey sacs were diluted with water, and a quantity of 80 microliters was inoculated on MYP agar.

P. larvae colonies were easy to distinguish macroscopically. However, to exclude any doubt, the Catalase test was used. Only the colonies which were macroscopically identified as *P. larvae* colonies and were Catalase negative were counted as *P. larvae* colonies.

2.9 Determination of the concentration of *P. larvae* spores in honey sacs through the growth of its colonies on MYP agar after various periods of digestion

From the basic sugar solution, with the known concentration of *P. larvae* spores, (providing the growth of 50 *P. larvae* colonies on MYP agar), three other dilutions were made: n/2, n/4 and n/8.

After that, 5 groups of 21 honey bees were individually isolated and after starvation, fed *ad libitum*. Four groups of bees were fed with sugar solutions containing various concentrations of *P. larvae* spores and one group was used as a control group and was fed with a pure sterile sugar solution.

From each group, 7 bees were killed after 15 minutes, 7 after 30 minutes, and after 60 minutes the last 7 bees were killed. Their bodies were stored in a refrigerated container and each honeybee was individually examined. Each honey sac was weighed, diluted with sterile water, treated by a hot water bath and 80 microliters of such a solution was inoculated in one Petri dish. As a control, 10 Petri dishes were inoculated with a pure sterile solution, and the other 10 with a basic sugar solution containing 'n' concentration of *P. larvae* spores.

After the incubation period of three days at 36^{O} C, *P. larvae* colonies were counted in each Petri dish. They were recognized macroscopically, and by the Catalase test.

3. RESULTS AND DISCUSSION

The level of honey consumption in honey sacs (during various periods of time) was 3.24 milligrams per bee after 10 minutes, after 20 minutes it was 6.15 milligrams per bee, after 30 minutes it was 7.17 milligrams per bee, after 60 min. it was 9.49 milligrams per bee, after 90 minutes it was 14.75 milligrams per bee, and after 120 minutes it was 17.35 milligrams per bee (Table 1).

Six hours after two groups of 30 honeybees were fed *ad libitum*, the average value of honey consumption was 23 mg per honeybee, and after 18 hours it was 42.10 mg per bee (Table 2).

Table 1. Honey consumption in honey sacs (during various periods of time)

Time	Honey sacs before (mg)	Honey sacs after (mg)	Consum. honey (mg)	Time	Honey sacs before (mg)	Honey sacs after (mg)	Consum. honey (mg)
10 min	40.00	35.70	4.30	60 min	43.60	33.10	10.50
	28.40	26.90	1.50		55.40	42.60	12.80
	24.00	21.80	2.20		19.60	10.10	9.50
	30.50	28.20	2.30		27.10	12.50	14.60
	20.10	17.90	2.20		12.10	4.50	7.60
	20.10	13.20	6.90		17.30	15.40	1.90
	41.50	37.70	3.80		38.70	27.60	11.10
	28.70	26.00	2.70		29.20	21.30	7.90
	35.90	32.70	3.20		43.70	34.40	9.30
	40.80	37.50	3.30		19.80	9.10	10.70
Average			**3.24**	Average			**9.67**
20 min	18.40	10.20	8.20	90 min	19.80	3.60	16.20
	26.30	22.20	4.10		21.10	8.60	12.50
	38.40	31.10	7.30		23.70	6.20	17.50
	31.70	26.70	5.00		35.00	25.10	9.90
	27.90	19.90	8.00		30.50	12.90	17.60
	42.80	38.50	4.30		32.40	19.60	12.80
	21.30	16.10	5.20		28.90	12.20	16.70
	19.40	12.30	7.10		34.70	21.00	13.70
	28.90	23.00	5.90		25.80	10.00	15.80
	25.40	19.00	6.40		20.40	5.60	14.80
Average			**6.15**	Average			**9.67**
30 min	28.80	20.60	8.20	120 min	38.00	24.80	13.20
	26.30	22.30	4.00		38.10	16.60	21.50
	43.90	40.50	3.40		34.90	13.70	21.20
	49.40	48.20	1.20		29.70	16.20	13.50
	26.50	7.10	19.40		35.10	18.00	17.10
	44.70	36.70	8.00		28.60	11.00	17.60
	27.80	21.60	6.20		31.40	12.50	18.90
	39.40	32.80	6.60		37.80	22.00	15.80
	28.50	20.90	7.60		39.00	23.70	15.30
	32.90	25.80	7.10		43.00	23.60	19.40
Average			**7.17**	Average			**17.35**

Source: designed by authors

Table 2. Honey consumption (controlled in honey sacs)

			6 HOURS			18 HOURS	
Number of groups	Number of bees	Food taken by 5 bees (mg)	Weight of honey sacs (per bee) (mg)	Consumed honey (mg)	Food taken by 5 bees(mg)	Weight of honey sacs (per bee) (mg)	Consumed honey (mg)
I	1	42.23	19.14	22.71	44.21	1.98	42.18
	2		20.51			1.87	
	3		18.34			1.84	
	4		19.85			2.31	
	5		19.76			2.15	
	Average		**19.52**			**2.03**	
II	1	44.65	20.54	23.54	42.85	0.76	42.05
	2		21.68			0.68	
	3		21.30			0.89	
	4		20.83			0.93	
	5		21.20			0.74	
	Average		**21.11**			**0.80**	
III	1	42.54	19.24	22.75	43.27	1.18	42.07
	2		20.14			1.14	
	3		19.85			1.32	
	4		20.58			1.04	
	5		19.14			1.32	
	Average		**19.79**			**1.20**	
IV	1	43.87	21.18	22.94	43.18	0.58	42.58
	2		21.05			0.63	
	3		20.73			0.59	
	4		20.89			0.61	
	5		20.80			0.59	
	Average		**20.93**			**0.60**	
V	1	41.98	18.71	23.09	42.74	0.78	41.88
	2		18.94			0.81	
	3		19.01			0.79	
	4		18.53			0.98	
	5		19.26			0.94	
	Average		**18.89**			**0.86**	
VI	1	42.75	19.73	22.97	43.18	1.41	41.84
	2		19.89			1.30	
	3		20.17			1.29	
	4		19.70			1.38	
	5		19.61			1.32	
	Average		19.82			1.34	
Average (mg/bee)			**23.00**			**42.10**	

Source: designed by authors

Quantities of honey delivered through social contacts:

When one fed honeybee was in contact with just one hungry bee, 27% of primarily consumed food was delivered to the hungry bee (each hungry bee received 7.02 milligrams honey). The results represent the average of 5 groups of honeybees with a ratio of 1 fed bee: 1 hungry bee.

When one fed honeybee was in contact with two hungry bees, 35% of primarily consumed food was delivered to hungry bees (each bee received 6.13 milligrams of honey). The results represent the average of 5 groups of honeybees with a ratio of 1 fed bee: 2 hungry bees.

When one fed honeybee was in contact with three hungry bees, 43% of primarily consumed food was delivered to the hungry bees (each bee received 4.70 milligrams of honey). The results represent the average of 5 groups of honeybees with a ratio of 1 fed bee: 3 hungry bees.

When one fed honeybee was in contact with four hungry bees, 48% of primarily consumed food was delivered to the hungry bees (each bee received 4.44 milligrams of honey). The results represent the average of 5 groups of honeybees with a ratio of 1 fed bee: 4 hungry bees.

When one fed honeybee was in contact with five hungry bees, 50% of primarily consumed food was delivered to the hungry bees (each bee received 3.60 mg of honey). The results represent the average of 5 groups of honeybees with a ratio of 1 fed bee: 5 hungry bees (Table 3).

Table 3. Quantity of honey delivered through social contacts

Honey bees		Quantity of honey consumed by fed bees (mg)	SUM	Quantity of honey delivered from fed to hungry bees (mg)	SUM	Delivered honey through social contacts (%)	Average quantity of honey delivered to single bee (mg)
Fed	Hungry						
1	1	24.80		6.84			
1	1	27.10		7.68			
1	1	23.60	**130.00**	5.24	**35.10**	**27%**	**7.02**
1	1	26.20		7.19			
1	1	28.30		8.15			
1	2	35.80		15.84			
1	2	37.40		10.70			
1	2	31.94	**175.00**	14.01	**61.25**	**35%**	**6.13**
1	2	34.58		9.47			
1	2	35.28		11.23			
1	3	33.54		14.18			
1	3	31.65		15.14			
1	3	29.58	**164.00**	13.89	**70.52**	**43%**	**4.70**
1	3	35.12		12.57			
1	3	34.11		14.74			
1	4	37.14		18.23			
1	4	35.68		16.58			
1	4	31.94	**185.70**	17.53	**88.80**	**48%**	**4.44**
1	4	39.52		18.54			
1	4	41.42		17.92			
1	5	36.24		18.54			
1	5	37.58		18.27			
1	5	35.12	**180.10**	15.34	**90.00**	**50%**	**3.60**
1	5	32.87		17.89			
1	5	38.29		19.96			

Source: designed by authors

Transmission of *P. larvae* spores through social contacts was registered in all measurements:

An average of 48.33 *P. larvae* colonies grew on MYP agar after the extraction of honey sacs from the honey bees fed with a known concentration of *P. larvae* spores in a sugar solution (1 *P. larvae* colony = 119 ± 15 spores);

The number of *P. larvae* colonies grown after extracting the honey sacs from the bees after the first contact was 46.23, after the second contact it was 45.69, and after the third contact it was 45.07 colonies.

The results represent the average of 5 repeated experiments. In each experiment 5 groups of 4 single honeybees were used (one fed bee and three hungry bees).

Concentration of *P. larvae* spores in honey sacs after various periods of digestion was mostly constant:

a) The number of *P. larvae* colonies (grown on MYP agar) from extracted honey sacs of the honeybees fed with a sugar solution with a known concentration of *P. larvae* spores (n), was 49.75, after 15 minutes, 49 after 30 minutes, and 51 after 60 minutes (1 *P. larvae* colony = 119 +- 15 spores).

b) The number of *P. larvae* colonies (grown on MYP agar) from the extracted honey sacs of the honeybees fed with a diluted sugar solution with n/2 concentration of *P. larvae* spores, was 25 after 15 minutes, 25.8 after 30 minutes, and 25 after 60 minutes.

c) The number of *P. larvae* colonies (grown on MYP agar) from the extracted honey sacs of the honeybees fed with a diluted sugar solution with n/4 concentration of *P. larvae* spores, was 12.75 after 15 minutes, 12.67 after 30 minutes, and 12 after 60 minutes.

d) The number of *P. larvae* colonies (grown on MYP agar) from the extracted honey sacs of the honeybees fed with a diluted sugar solution with n/8 concentration of *P. larvae* spores, was 6.33, after 15 minutes, 6.25 after 30 minutes, and 6.25 after 60 minutes.

The results represent the average of 7 honey bees for each concentration (n, n/2, n/4, n/8) and measurement of time (15 min, 30 min, 60 min).

The number of *P. larvae* colonies in agar inoculated with pure sterile sugar solution was negative and in agar inoculated with the material containing 'n' concentration of *P. larvae* spores 48 to 50 *P. larvae* colonies grew in one dish.

The spreading of *P. larvae* infection within a colony of honeybees started when honeybee larvae were infected, while consuming spores present in their food. The spores germinated in the lumen of the intensin, liberating the vegetative rods, which entered the body cavity and proliferated in the hemolymph (Bailey, 1981; Bailey, Ball 1991). Only larvae less than two days old were readily infected, they died in either the larval or pupal stage and each infected larvae produced about 2.5×10^9 spores.

A cell-cleaner bees became contaminated with *P. larvae* spores, while trying to remove the infected brood out of the hive. *P. larvae* infection could be spread either to larvae reared in a cell which previously contained an infected larvae or cell-cleaner bees might transmit spores to larval food when they became nurse bees.

From the results of the experiments, it was obvious that quantity of honey delivered from one single honeybee to one or more hungry bees depended on the content of its honey sac and on the number of hungry bees. The total amount of honey delivered through social contacts from one fed bee to just one hungry bee was 27%, but if the bee was in contact with 5 hungry bees, it delivered 50% of the honey to the hungry bees.

Consequently, when more bees were involved, each of them received a lower quantity of honey through social contacts.

Repeated social contact did not significantly influence the level of concentration of *P. larvae* spores. In the examined honeybees even after three repeated contacts, the concentration of *P. larvae* spores in their honey sacs remained relatively constant.

When the honeybees were fed *ad libitum*, they filled their honey sacs, and they digested about 3 mg of honey after 10 min, 17 mg after 2 hours and 42 mg after 18 hours, which was almost all they consumed.

The concentration of *P. larvae* spores in the honey sacs was not significantly different during various times of digestion which meant that in spite of the fact that the volume of the honey sacs was smaller after some time had pas0sed, the concentration of the *P. larvae* spores remained more or less constant. The obtained results were similar when the bees were fed with different concentrations of *P. larvae* spores in their food.

4. CONCLUSION

The results of the experiments showed that the quantity of honey delivered from one single honeybee to one or more hungry bees depended on the content of its honey sac and on the number of hungry bees. The total amount of honey delivered through social contacts from one fed bee to just one hungry bee was 27%, but if the bee was in contact with 5 hungry bees, it delivered 50% of the honey to the hungry bees.

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PRIJENOS SPORA *PAENIBACILLUS LARVAE* SOCIJALNIM KONTAKTIMA UNUTAR PČELINJE ZAJEDNICE *(APIS MELLIFERA CARNICA POLLMAN, 1879)*[4]

SAŽETAK

Američka gnjiloća pčelinjeg legla široko je rasprostranjena, vrlo tvrdokorna pčelinja bolest, uzrokovana sporoformnom bakterijom Paenibacillus larvae*. Bolest je vrlo zarazna, te može uzrokovati velike gubitke u pčelarstvu, ako se ne uoči u ranoj fazi. Cilj ovog rada bio je istražiti što se događa s razinom koncentracije spora* P. larvae *tijekom socijalne izmjene hrane i tijekom različitih razdoblja digestije sporama zaražene hrane u mednim mjehurima pčela. Metoda za određivanje prisutnosti i broja spora* P. larvae *u uzorcima meda koju su opisali Ritter i Kiefer (1993) bila je modificirana tako da se iz broja izraslih kolonija* P. larvae *na MYP agaru omogućilo određivanje koncentracije spora* P. larvae *u mednim mjehurima pojedinih pčela. Bez obzira na količinu socijalnim kontaktima izmijenjene hrane, poznata koncentracija spora* P. larvae *ostala je nepromijenjena. I nakon različitih razdoblja digestije, koncentracija spora* P. larvae *bila je konstantna. Dokazano je da pčele nemaju mogućnost smanjenja broja spora* P. larvae *filtracijom kroz medni mjehur.*

Ključne riječi: Paenibacillus larvae*, spore, filtracija, medni mjehur*

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