

## IS IT POSSIBLE TO INTRODUCE *TROPILAEALAPS CLAREAE* TOGETHER WITH IMPORTED HONEY BEE QUEENS TO EUROPE?

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### S u m m a r y

We investigated the life span of adult *T. clareae* mites on adult *Apis mellifera* workers and queens. In the first series, each cage contained 1 queen, 9 workers and 10 mites. In other series we put 1 queen and 2 mites into group T1 and from 2 to 7 *T. clareae* mites into other groups (T2-T7) with a queen and with 1 to 6 attendance workers. Numbers of dead mites were recorded every 12 hours in each series. The tubes and cages with last surviving mites were observed every 2 hours to designate the maximal life span of *T. clareae* mites. The average life span of 780 *T. clareae* mites existing on the adult bees were calculated.

Most mites died during the first 12 hours in both experimental groups kept in plastic cages with a queen and 9 worker bees - in an incubator (53 mites), and in room temperature (39 mites), and in other experimental groups - in the plastic cage (33 mites), and in wooden one (39 mites) with a queen and 9 worker bees in room temperature. The differences of the average life span of *T. clareae* mites in plastic cages depended on the number of attendance worker bees from 18.5 h in T5 (with a queen, 5 workers and 6 mites) to 28.2 h in T2 (with a queen, 1 worker and 2 mites). The maximal life span was different, too - from 44.5 h in the group T4 (with a queen, 3 workers and 4 mites) to 59.1 h in T7 (with a queen, 6 workers and 7 mites).

The studies demonstrate that *T. clareae* can not survive more than 74 h on adult workers and queens. Considering a long shipment time from Nepal to Poland (minimum 65 h) and very low percentage of mites which live longer than 60 h, we can say that it is impossible to introduce *T. clareae* mites together with imported bee queens into European apiaries. To be sure not to introduce *T. clareae* together with imported queens to Europe it is enough to wait 70 h since the time of packing.

**Keywords:** *Tropilaelaps clareae*, queen, honeybee, *Apis mellifera*, life span of *T. clareae* on workers and queens.

### INTRODUCTION

There was a discussion all over Poland in 1999 whether to import or not mated queens from Nepal to replace queens in overwintered colonies in early spring (Wilde 1999, Wilde and Wilde 1999). The main point of that discussion was the question - is it dangerous for Polish and European

beekeeping or not? Was it possible to bring different bee diseases with queens to Europe? It was the *T. clareae* mite which was mostly mentioned.

Woyke estimated (1984) that mites could survive on adult bees only 2-3 days. Contrary, the recent research (Woo 1999) revealed that adult *T. clareae* could survive for 5-7 days without a host. Woyke (1985) ascertained that *T. clareae* was a serious pest for *Apis mellifera* in the tropics only, but it was not dangerous in our (European) temperate zones. It is known that *Varroa jacobsoni* has a few different subspecies, and according to Anderson (2000), even species. Can we speak about the same phenomenon of *T. clareae*, too? Or the differences in both experiments mentioned above resulted from different condition during the investigations only?

The main aims of our experiment were to find out why there are such big differences in both cited above experiments and to get the answer to the question mentioned in the topic of this paper.

## MATERIALS AND METHODS

The investigation was carried out in Apiculture Centre in Nepal, located 120 km from Kathmandu (Chitwan District) and lasted from February to June 2000. We investigated the life span of adult *T. clareae* mites on adult *Apis mellifera* workers and queens, four series of observations were conducted.

In the first series we put 9 mites and 3 workers into plastic tubes (ca. 30 ml). The mites were collected from cells with pupae. We put one group (5 tubes) into an incubator (34°C) and we kept another one in room temperature (21-25°C).

In the second one 10 *T. clareae* mites were introduced into shipment cages with a queen, 9 attendant workers and a piece of candy. We put 10 cages into an incubator and 10 kept in room temperature.

In the third series we compared mite survival in plastic and wooden cages in room temperature only. Each cage contained a queen and the 9 workers and 10 mites.

Finally in the fourth series, plastic cages with a queen and different number of workers and mites were observed. We put 1 queen and 2 mites into group T1 and from 2 to 7 *T. clareae* mites into other groups (T2-T7) with a queen and from 1 to 6 attendant workers.

Numbers of dead mites were recorded every 12 hours in each series. The tubes and cages with last surviving mites were observed every 2 hours to designate the maximal life span of *T. clareae* mites. The average life span of 780 *T. clareae* mites on adult bees were calculated.

Significance of differences in average life span of *T. clareae* mites was determined by variance analysis (ANOVA) and multiple range test with least

significant difference range (LSD). The values which differ significantly at  $p = 0.01$  are indicated with capitals.

## RESULTS

Table 1 shows that most mites died during the first 12 hours in an incubator while in room temperature within 24 to 36 hours. The average life span of *T. clareae* mites in an incubator was shorter (20:42 h) than in room temperature but the differences were not significant. The maximal life span was 22:22 h shorter in an incubator (47:14 h) than in room temperature (69:36 h).

Table 1

Survival rate of *Tropilaelaps clareae* in plastic tubes (sum of 5 tubes in each group)\* - Przeżywalność *Tropilaelaps clareae* w pojemnikach plastikowych (suma 5 pojemników w każdej grupie)\*

Time in h Czas w godz.	Number of dead mites - Liczba martwych roztoczy	
	Incubator - Cieplarka	Room temperature Temperatura pokojowa
0-12	16	11
12-24	9	7
24-36	14	18
36-48	6	5
48-60	0	3
60-72	0	1
Total mites Roztoczy ogółem	45	45
Average of life span $\pm$ s Średnia długość życia $\pm$ s	20:42 $\pm$ 2:54	26:01 $\pm$ 5:36
Max. life span Max. długość życia	47:14	69:36

x / in each plastic tube (volume 30 ml) 9 mites with 3 worker bees - w każdym plastikowym pojemniku o pojemności 30 ml było 9 roztoczy z 3 robotnicami; s = standard deviation; s = odchylenie standardowe

Table 2 shows that most mites died during the first 12 hours in both experimental groups - in an incubator (53 mites), and in room temperature (39 mites). The average life span of *T. clareae* mites in an incubator was significantly (at  $p = 0.01$ ) shorter (17:02 h) than in room temperature (26.21 h). The maximal life span was 28:48 h shorter in an incubator (44.43 h) than in room temperature (73:31 h).

Table 3 shows that most mites died during the first 12 hours in both experimental groups - in the plastic cage (33 mites); and in wooden one (39 mites). The average life span of *T. clareae* mites in plastic and wooden cages

was similar - 25:49 and 23:36 h, respectively, likewise the maximal life span - 58:12 and 57:37, respectively.

Table 4 shows that worker bees in cages in room temperature (from 0 in group T1 to 6 in group T7). Most mites died during the first 12 hours in 4 experimental groups - T1 (8), T3 (11), T5 (19) and T6 (21). In other 3 groups most mites died from 12 to 24 hours - 6, 19 and 27 in T2, T4 and T7 respectively. The average life span of *T. clareae* mites in plastic cages varied between 18:28 h in T5 (a queen, 5 workers and 6 mites) to 28:12 h in T2 (a queen, 1 worker and 2 mites). But there were no significant differences. The maximal life span varied, too - from 44:31 h in group T4 (a queen, 3 workers and 4 mites) to 59:08 h in T7 (a queen, 6 workers and 7 mites).

Table 2

Survival rate of *Tropilaelaps clareae* in plastic cages  
(sum of 10 cages in each group)\* - *Przeżywalność Tropilaelaps clareae* w  
klateczkach plastikowych (suma 10 klateczek w każdej grupie)\*

Time in h Czas w godz.	Number of dead mites - Liczba martwych roztoczy	
	Incubator - Ciepłarnia	Room temperature Temperatura pokojowa
0-12	53	39
12-24	19	13
24-36	11	9
36-48	17	21
48-60	0	15
60-72	0	3
Total mites Roztoczy ogółem	100	100
Average of life span $\pm$ s Średnia długość życia $\pm$ s	17:02 <sup>A</sup> $\pm$ 3:42	26:21 <sup>B</sup> $\pm$ 4:44
Max. life span Max. długość życia	44:43	73:31

x / in each plastic cage 10 mites with queen and 9 worker bees - w każdej klateczce plastikowej było 10 roztoczy, matka i 9 robotnic; s = standard deviation; s= odchylenie standardowe; Different capitals indicate significant differences at p = 0.01 - Różne duże litery oznaczają różnice istotne przy p = 0,01

Table 3

Survival rate of *Tropilaelaps clareae* in plastic (PC) and wooden cages (WC)  
(sum of 10 cages in each group) x - Przeżywalność *Tropilaelaps clareae* w  
klateczkach plastikowych (PC) i drewnianych (WC) (suma 10 klateczek w  
każdej grupie)\*

Time in h Czas w godz.	PC	WC
0-12	33	39
12-24	14	16
24-36	19	12
36-48	23	25
48-60	11	8
Total mites Roztoczy ogółem	100	100
Average of life span $\pm$ s Średnia długość życia $\pm$ s	25:49 $\pm$ 5:24	23:36 $\pm$ 4:55
Max. life span Max. długość życia	58:12	57:37

x / in each cage 10 mites with queen and 9 worker bees - w każdej klateczce było 10 roztoczy,  
matka i 9 robotnic; s = standard deviation; s = odchylenie standardowe

Table 4

Survival rate of *Tropilaelaps clareae* depending upon number of attendant  
worker bees (sum of 10 cages in each group) x

	Group - Grupa						
	T1	T2	T3	T4	T5	T6	T7
Number of workers Liczba robotnic	-	1	2	3	4	5	6
Number of mites Liczba roztoczy	2	2	3	4	5	6	7
Time in h Czas w godz.	Number of death mites - Liczba martwych roztoczy						
0-12	8	3	11	10	19	21	17
12-24	5	6	7	19	13	16	27
24-36	2	4	6	7	15	14	18
36-48	3	5	5	4	3	6	4
48-60	2	2	1	0	0	3	4
Total mites Roztoczy ogółem	20	20	30	40	50	60	70
Average of life span $\pm$ s Średnia długość życia $\pm$ s	21:36 $\pm$ 4:05	28:12 $\pm$ 5:02	21:12 $\pm$ 4:41	19:29 $\pm$ 3:12	18:28 $\pm$ 3:54	20:49 $\pm$ 4:50	21:36 $\pm$ 2:57
Max. life span Max. długość życia	49:19	54:12	58:06	44:31	46:49	57:02	59:08

x/ each plastic cage contains a queen - w każdej klateczce była matka

## DISCUSSION

According to Woyke (1984) most *T. clareae* mites can survive on adult worker bees up till 2 days. We got the same result keeping *T. clareae* mites on adult workers and queens in an incubator (table 1 and 2). However we found some mites which survived over 3 days in room temperature (table 2) which is similar to results achieved by Koeniger and Muzaffar (1988). The differences of mites' life span between room and incubator conditions are quite similar to Woyke's (1984) results who got a few alive mites after 2 days in room temperature only. He did not investigate a survival rate of *T. clareae* in an incubator, but only in a hive. Though we can assume that the hive condition of Woyke's experiment are similar to ours in an incubator. Why did we observe some mites which survived even 3 days in room temperature, while in Woyke's experiment (1984) they lived only 60 h? I think that the main reason of the difference was the different number of *T. clareae* mites which were investigated. Woyke observed 63 mites on adult workers and 41 mites on the comb with eggs only. In second series on adult workers he got 1 mite which survived 60 h. It was less than 1% (0.96) of all observed mites and 2.8% of mites observed in room condition. We observed 780 mites in our experiment. And we got only 4 *T. clareae* mites which survived more than 60 h of all of them. It was 0.51% only. So we can not eliminate that he could have found a few mites with longer life span if he had examined a larger number of *T. clareae* mites. However, it is totally unclear how Woo (1999) got 5-7 days survival of *T. clareae* mites without a host, the more so, because the condition of his experiment are quite unknown. We have never observed mites which survived more than 48 h without a host (Wilde 2000).

We did not find any significant differences in life span of *T. clareae* mites in plastic and wooden cages and comparing different number of attendant bees in cages. So we can conclude that *T. clareae* mites can not survive more than 74 h on adult honeybee workers or queens of *A. mellifera*. However, the results achieved by Rinderer et al (1994) show the survival of mites, whether on *A. mellifera* or *A. dorsata* about one day longer than reported by Koeniger and Muzaffar (1988) and Woyke (1984). He suggested also that different, less artificial conditions would support still longer periods of *T. clareae* survival.

Is it enough time not to introduce mites to Europe with exported queens from Nepal? We need at least 8-10 h to pick up the queens from mating boxes and put them into shipment cages, indeed, usually we did it 20-24 h before shipment. We need next 6-8 h to bring the packages with queens from Dabur Apiculture Center to the airport in Katmandu, which has to be finished 5-6 h before the airplane departure. The fastest way to bring the queens to Poland is via Moscow (AEROFLOT) which takes 26 h (together with waiting for the

next airplane from Moscow to Warsaw). Custom official formality in Poland takes at least 8 h. The way to the place where the queens can be examined by Veterinary Officials takes next 6 h. So it takes all together minimum 65 h from the pick up time of the queens. To be sure not to introduce *T. clareae* mites into honeybees colonies in Poland we ought to wait 5 h more before we start queens replacement.

Therefore we advise to know at what time exactly packing of the last series of queens in each shipment was ended and not to sell the queens until 70 h pass since the time of packing.

## CONCLUSION

The studies demonstrate that *T. clareae* can not survive more than 74 h on adult workers and queens. To be sure not to introduce *T. clareae* together with imported queens to Europe it is enough to wait 70 h since the time of packing.

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# CZY MOŻLIWE JEST WPROWADZENIE DO EUROPY TROPILAEALAPS CLAREAE WRAZ Z IMPORTOWANYMI MATKAMI?

Wilde J.

## S t r e s z c z e n i e

Badano długość życia dorosłych roztoczy *T. clareae* na robotnicach i matkach *Apis mellifera*, znajdujących się w pojemnikach plastikowych i klateczkach wysyłkowych w cieplarni i temperaturze pokojowej. Każda klateczka w serii 1. (plastykowa i drewniana) zawierała matkę i 9 robotnic oraz 10 roztoczy. Obserwowano też długość życia roztoczy w zależności od liczby pszczoł towarzyszących matce w klateczce. Utworzono grupę z matką w klateczce bez pszczoł towarzyszących, do której poddano 2 roztocze (T1) oraz od 2 do 7 roztoczy *T. clareae* do pozostałych grup (T2-T7), z matką i z 1 do 6 robotnicami.

Przeżywalność roztoczy określano, co 12 godzin. W celu oznaczenia max. długości życia, pojemniki i klateczki z ostatnimi żyjącymi roztoczami kontrolowano, co 2 godziny. Określono średnią długość życia 780 roztoczy *T. clareae* poddanych na dorosłe pszczoły. Średnia długość życia roztoczy *T. clareae* w cieplarni była krótsza (o 20:42 godz.), niż w temperaturze pokojowej, choć nie stwierdzono różnic statystycznie istotnych. Najwięcej roztoczy w klateczkach plastikowych z matką i 9 robotnicami padło w ciągu pierwszych 12 godz., bez względu na to, czy były przetrzymywane w cieplarni (53 roztoczy), czy w temperaturze pokojowej (39 roztoczy). Średnia długość życia roztoczy *T. clareae* w cieplarni (17:02 godz.) była wysoko istotnie krótsza, niż w temperaturze pokojowej (26:21 godz.). Najwięcej roztoczy przetrzymywanych w temperaturze pokojowej padło podczas pierwszych 12 godz. obserwacji zarówno w klateczkach plastikowych (33 roztoczy), jak i drewnianych (39 roztoczy). Średnia długość życia roztoczy *T. clareae* w klateczkach plastikowych i drewnianych była podobna i wynosiła odpowiednio 25:49 i 23:36 godz., podobnie jak i max. długość ich życia - odpowiednio 58:12 i 57:37 godz.

Według Woykego (1984) roztocze *T. clareae* na dorosłych osobnikach pszczelich mogą żyć najwyżej 2,5 dnia. W naszym doświadczeniu otrzymaliśmy podobne rezultaty jedynie w cieplarni (tabela 1 i 2). Zaobserwowaliśmy jednakże, iż w temperaturze pokojowej niektóre roztocze żyły ponad 3 dni. Woyke obserwował życie 63 roztoczy na robotnicach i 41 na plastrze, w komórkach z jajami. Było to zaledwie 0,96% wszystkich badanych przez niego roztoczy, a 2,8% obserwowanych w temperaturze pokojowej. W naszych badaniach obserwowaliśmy 780 roztoczy, uzyskując tylko 4 osobniki żyjące ponad 60 godz., co stanowiło zaledwie 0,51%. Nie można więc wykluczyć, iż autor ten mógłby znaleźć kilka roztoczy żyjących dłużej, gdyby przebadał większą ich liczbę.

Na podstawie przeprowadzonych badań, można stwierdzić, iż roztocze *T. clareae* nie mogą żyć dłużej niż 74 godz. na matkach i pszczołach towarzyszących, w klateczkach wysyłkowych. Biorąc pod uwagę długi okres związany z importem matek z Nepalu do Polski (min. 65 godz.) i bardzo małym odsetkiem roztoczy żyjących dłużej niż 60 godz. można powiedzieć, iż wątpliwe jest wprowadzenie ich do pasiek Europy, wraz z importowanymi matkami. Aby mieć całkowitą pewność, iż nie poddamy żywych roztoczy wraz z matkami w Europie wystarczy odczekać 70 godz. licząc od momentu pakowania matek do klateczek w Nepalu.

**Słowa kluczowe:** *Tropilaelaps clareae*, matka, pszczoła miodna, *Apis mellifera*, długość życia *T. clareae* na robotnicach i matkach.