SEASONAL CHANGES IN MITE (TROPILAELAPS CLAREAE) AND HONEYBEE (APIS MELLIFERA) POPULATIONS IN APISTAN TREATED AND UNTREATED COLONIES

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ABSTRACT

Beekeepers in Pakistan in 1992 repeatedly complained of heavy losses of honey and honeybee colonies owing to the disease problem mainly caused by *Tropilaelaps clareae*, Asian parasitic brood mite. Despite frequently treating their colonies with low-cost chemicals, like sulphur, tobacco and camphor (naphthalene), many colonies were still found infested. In order to achieve a better control of the parasite, it was essential to understand the biology and behaviour of mite.

This paper describes the development of *T. clareae* infestation in the Apistan treated and untreated *A.mellifera* colonies having brood and foraging through the year under Islamabad and Chakwal conditions in Pakistan covering a period of 14 months since October 1993. The study was carried out using *A.mellifera* colonies in group A and B that were naturally infested with T. clareae. Group A: A set of four low-infested colonies treated with Apistan. Group B: A set of four highly infested colonies remained untreated.

Observations on *T. clareae* population fluctuations in eight colonies were recorded by regular monitoring of weekly hive debris. Mean infestation level in bees and brood (mites/100 bees and mites /100 brood cells) were determined bimonthly. The colonies have mostly remained in Islamabad except for moving them once to Chakwal for a period of 6 weeks for the third honey crop during September-October 1994. The highest mite density was recorded in debris for untreated group B in March 1994 followed by sudden decrease in April and gradual decrease reaching minimum in September. The mite infestation in brood peaks in May and October coinciding with increased brood rearing activity in *A. mellifera* colonies in Islamabad and Chakwal. *T. clareae* re-infests the treated colonies during the active honey flow periods, that is during April and in October. These observations helped to explain differences in the level of damage with least and heavy infestations caused to beekeeping with *A. mellifera* in Pakistan.

INTRODUCTION

The parasitic honeybee mite, *T. clareae*, causes severe damage to beekeeping with *A. mellifera* in Pakistan. To control it the biology or the development of the mite populations must be studied. This would provide us with information about the establishment of the interlinkage of the host and the parasite and could lead to improvement, of timing, in dosage determination and methods of either chemical or biotechnical control.

This paper describes the development of *T. clareae* infestation in the treated and untreated *A. mellifera* colonies having brood and foraging through the year under Islamabad and Chakwal conditions in Pakistan covering a period of 14 months. The colonies have mostly remained in Islamabad except for moving them once to Chakwal for a period of 6 weeks for the third honey crop during September-October. These observations helped to explain differences in the levels of damage with least and heavy infestations caused to beekeeping with *A. mellifera* in Pakistan.

MATERIALS AND METHODS

This study was carried out at the National Agricultural Research Centre (NARC) over a period of 14 months. in Islamabad, Pakistan, using *A. mellifera* colonies that were naturally infested with *T. clareae*. Periodic examinations of mite populations were made in brood, adult bees and debris samples in two sets of colonies. The mite infestations had been observed under controlled and treated conditions since October 1993. A set of four low-infested colonies were treated with Apistan in November 1993 and then in June 1994 in group A, while the four highly infested colonies in group B remained untreated. The colonies were not equalized at the start of the experiment. They were allocated randomly. The two groups were placed in the apiary at a distance of 2 m from one another but with entrances facing in different directions to minimize the movement of bees between colonies.

Equalization in terms of queen (one year old) was undertaken. Equalization of all other parameters was not possible if cross-infestation was to be avoided.

The following observations were taken for each colony:

<u>Strength of the colonies</u> : The strength of the colonies was determined by estimating the number of bees and measuring the brood area on each frame in the last week of every month on sunny days when bees were actively foraging.

<u>The diagnosis of mites</u> : The infestation level, that is the number of parasites in each colony, was estimated as follows.

Samples of bees and brood (200-300 adult bees; 100 sealed brood cells) were taken after every two months, and the number of mites found on them. Characterization of the postembryonic stages of the female castes of the honey bee, *A. mellifera* L, was determined using the system developed by Rembold & Kremer, (1980) and Jay (1962). The brood comb samples were previously frozen before examining. The identification of the mite stages was based on Delfinado Baker & Baker, (1985).

Sampling weekly debris on white formica sheets, which were placed in the hives and examined for mite mortality.

With these observations, the seasonal growth of both bees and mite population dynamics in 4 untreated colonies and 4 colonies under chemotherapy (Apistan) was determined. The analysis is given in Tables 1-5. Mean infestation levels in bees and brood were (mites/100 bees and mites/100 brood cells) determined.

<u>Honey harvest</u> : Honey was harvested from all colonies at the end of each honey flow to compare the honey productivity of the treated and untreated colonies (Table 4).

RESULTS

SEASONAL GROWTH IN BROOD, BEES AND MITE

The seasonal growth of brood and bees in Group A is comparatively better than Group B (Fig 1&2). The measurement of sealed brood in colonies and the mites, ability to reproduce was examined during the five seasons of a beekeeper's year at NARC, Islamabad. The results show that brood rearing is more or less continuous throughout the year. A normal colony increases its brood rearing from January going to a maximum in May (1994), it decreases twice a year, once in September after the monsoon and the second time in December in winter. The two small peaks of brood rearing are in July and October. The reason is probably the climatic change and the honeybee flora.

LIFE CYCLE AND HABIT OF T. CLAREAE IN UNTREATED COLONIES

In untreated infested bee colonies *T. clareae* begins to emerge increasingly in early spring, with a population peak in debris in March, coinciding with the end of the oilseed rape, deciduous and citrus orchards flowering season and the beginning of the wild *Acacia* flowering season (Table 1-3). The bee population rises in May and June, the mite population decreases in the debris samples, however, the infestation rises inside the brood. There were mite infestation peaks in brood in May in summer, and also in autumn (October) in Chakwal (honey flow season). The maximum infestation of mites on young adult bees was 1.43± ranged from 0- 2.85% in March 1994, which means the mites use bees for dispersal during March. Drifting occurs from colonies with infestation to treated colonies free from mite.

Mites appear to reproduce throughout the year inside the brood in untreated Group B. The colonies reduce their brood rearing early in winter during December and second time late in summer in June. This reduction in brood rearing affects the reproduction of the mite. The average mite infestation reduced from 13.6% in November 1993 to 3.97% in brood sample in January 1994 in winter. Similarly it reduced from 14.01% in May to 5.7% in July 1994 in extreme hot and humid condition and then rose to 10.2% in October, 1994.

In the present study, it was observed that colonies increase brood rearing twice in a year. Once in May and a second time in October. With increase in brood rearing, there is increase in mite infestation, which clearly indicates that T. clareae mites can survive and thrive continuously given the provision of open bee (four-day-old larvae) brood of *A. mellifera* in abundance for successful fecundity(Woyke, 1994).

The initiation of active brood rearing starts in all colonies in Islamabad in January going upto maximum in May. This is determined by the climate and bee flora. Thus mites can reproduce more successfully in infested colonies if they produce brood earlier and for longer periods. The mite continues to breed during winter (October, November and December) in limited brood. The maximum downfall of winter mites in debris occurred in December (352 mites) in infested colonies. When pollen and nectar flow starts in January and February the number of reproducing females increase many folds. The mean natural mortality of mites in debris increases through February 754 mites reaching to a maximum of 3584 mites in March. (Table 1). The mean infestation in the brood decreases in July-August, when brood rearing is on the decline, pollen supply is short and there is high humidity. These unfavourable conditions discourage the reproduction of the mite in the brood as also observed in case of *V. jacobsoni* (Kraus & Velthius, 1997).

The results of this research have given, for the first time, a background to justify reasons why beekeepers in Pakistan have to constantly treat their colonies after a few days as the mite reproduces continuously in the colony having peaks and troughs depending on the quality and quantity of food coming into the colony under various seasons which increases and decreases the number of brood cells. The growth is attributed to the abundance of sealed brood.

There is a natural decline in mite mortality in debris samples after June, in the July-August (monsoon) season decreasing to a minimum in September.

The increase in infestation rate during October-November is due to the moving of the experimental colonies from Islamabad to a new location, Chakwal, for honey flow for a 5-6 week period. The ectoparasitic mite *T. clareae* therefore completes more reproductive cycles and mite population increases in untreated colonies.

POPULATION DYNAMICS OF *T. CLAREAE* IN APISTAN TREATED COLONIES

Colonies in the treated group A do not show any sign of infestation in brood nor on bees until early April when some dead mites were counted on the debris sheets and the first infestation or re-infestation of the treated colonies was determined. The first signs of damage was postponed for approximately five months from November 1993 when the colonies were first treated, to May 1994 when brood and bees samples were found heavily infested. The mean mite infestation in brood was 8.26% in May in Apistan treated colonies of group A. These colonies were re-infested in April, and the mite reproduced in two months reaching a maximum infestation in brood and bees in May. The colonies were treated with Apistan in June after the honey flow from clover tocontrol the mite. A maximum of 1647 dead mites were recorded during June. in these colonies under the affect of treatment.

DISCUSSION

There are few published research works on the natural history of *T. clareae* probably because the mite is presently limited to Asia and most research on parasitic mites is focussed internationally on *V. jacobsoni*. Within the last three decades, *V. jacobsoni* has spread to all continents except Australia and has been a subject of concern to beekeepers and scientists in all parts of the world.

Seasonal population dynamics

Observations by Aggarwal (1988) in *A. mellifera* colonies by regular monitoring of hive debris from November 1983 to June 1985, showed highest infestation during April 1984 with a minimum in July 1984 and March 1985 .

The parasitic bee mite *T. clareae* is considered to be more dangerous for *A. mellifera* than is *V. jacobsoni*. Differences due to climate in infestation of honey bee colonies by *T.clareae* mite under treated and untreated conditions have not been previously recorded in one area, however, infestation by *T.clareae* has been compared for different countries like Kabul and Vietnam (Woyke, 1987).

The mites were first noted in *A. mellifera* colonies in 1981 and has apparently spread through the beekeeping regions of the country by 1991 as the colonies are moved in large concentration to major honey flow periods in Islamabad and Potowar region. It was also documented in *A.cerana* in 1982 from samples provided from Islamabad in 1981 (Delfinado and Baker, 1982). By 1991 it has apparently spread through out the beekeeping regions of the country where beekeeping is practised as the colonies are moved throughout the country where good honey crop is available. There are reports of extensive colony mortality due to T. clareae in Afghanistan neighbouring country on the north west of Pakistan (Woyke, 1984).

The Pak-German Apiculture Promotion Project (PGAPP) Islamabad commissioned a study incorporating a survey, on beekeeping communities in northern Punjab and NWFP to solve and help out any problems regarding beekeeping. According to this survey carried out during 1991-92 about the bee health situation, it was revealed that *T.clareae* causes severe damage to honey bee colonies, resulting in loss of honey and massive colony mortality in Autumn. But the beekeepers and scientists cannot really make out the real loss because the results are mixed because of migratory beekeeping, Some beekeepers and a scientist at Bee Institute claim that *T. clareae* is not a big threat and can be managed, yet the effect on beekeeping has varied greatly in different parts of the country and different beekeeping practice (Stationery and migratory beekeeping, modern colony management with standard hives and equipment).

Parameters like low and high rates of brood and adult infestation have been investigated in two groups. These observations on colony development and mite reproduction may help in understanding the behaviour of the mite which may help to control it and also indicate which parameters are responsible for more rapid build up of populations of *T.clareae* in particular.

Along with the genetic factors, brood temperatures can have an influence upon the development of brood and therefore upon the length of sealing time. Since, as a rule, significantly more worker brood are raised than drone brood, in Pakistani conditions the development of the *T. clareae* mite population tends to correlate more with the cycle of the worker brood. All factors that lead to an increase of the brood capacity also lead to an increase of the mite population. Important factors include extreme climatic conditions such as heat, humidity and cold when decrease in brood rearing was observed.

The environment inside a hive is largely controlled by the bees and is fairly stable, especially the temperature in the brood nest, however factors such as humidity and food supply vary considerably according to the local and seasonal conditions, and these may also influence the growth of mite populations. Furthermore, the reaction of the bees to seasonal changes may directly or indirectly affect the mites. The low-infested treated colonies became infested after the growing phase in March was over and the adverse effect on brood reaching maximal in May did not affect the honey yield (Table 6).

As a rule, there is no hibernation in winter. The extent of brood is determined mostly by the amount of pollen and nectar available, which appears in January from *brassica* crops, in Pakistani winter in Islamabad region when brood rearing start increasing. The number of mites which infest a colony entering the main brood rearing period is therefore variable. The number is determined mainly by the brood rearing capacity at the beginning and end of a brood pause or least brood rearing which in Pakistani condition is December. The following are the five seasons experienced in Islamabad, Pakistan as reflected. by our data Winter = November-January (three months), Spring = February-April (three months), Summer = May-August (four months), Monsoon = mid-July-mid-August (one month), Autumn = September-October (two months).

Analysis of hive debris samples of this study revealed the presence of only one species of ecto-parasitic mite in them and that is *T. clareae* inflicting high infestation. The minimum downfall being 21mites for the month of September to maximum of 3584 mites for the month of March, 1994. Presence of high population of *T. clareae* in all the samples of infested colonies reveals that this mite has fully established itself as an ecto-parasite of *A. mellifera*. The presence of this mite in all the samples of treated colonies could be due to the fact that the nests of its original migratory host *A. dorsata* is seen to be found to settle on tree branches in late February and early March in Islamabad, which could enable the exchange of mite.

The absence of *V. jacobsoni* in brood and debris indicates that the mite has no interaction with *T. clareae* so there is no multiple mite parasitism in colonies in Islamabad. One such interaction between T. clareae and *V. jacobsoni* has been reported by Ritter (1987) in Thailand where at high population of T. clareae the *V. jacobsoni* population remained low.

Present study shows that population of *T. clareae* in Islamabad's sub-tropical climate grows exponentially during spring the first time, leaving the colonies at the verge of collapse. These colonies fail to give a honey harvest in the first crop (Table 6). The colony does not collapse immediately (one reason is that warmer climates enable honey bee colonies to produce a higher number of brood cycles per year than cold climates) but it survives the coming summer season because there is a continuous flow of two honey crops following one after the other in April and June which helps them to stabilise during April-June and these colonies give some honey. In June and July there is an increase in natural mite mortality observed in these colonies coming to a minimum in September. Factors such as mite mortality, grooming behaviour will lower mite population but not completely get rid of the infestation. As seen in present studies, *T. clareae* does not grow in number like *V. jacobsoni* does continuously growing in number. It has its peaks and troughs depending mainly on the amount of brood reared in the colony and temperature. It has its first sharp peak in March and then a steady decline in number in the summer, a second small peak in June. At the end of September when colonies are moved to the fourth nectar flow, the mite population starts to build up again the second time in the brood with the increase of brood rearing during October and a third increase in mite mortality observed in November. The growth of the mite declines again with the decrease in brood rearing in winter in December. Present study has shown mite population fluctuations during different seasons.

So maybe temperature in brood cells has a significant impact on the proportion of female offspring that reach adulthood within the brood cell and emerge together with the young bee.

If the infestation is not checked during December and then in June in the colonies there is a rapid build up of mite population during the favourable season of fresh pollen and nectar flow in January-February and October-November respectively, which stimulates an increase in honeybee brood rearing. The emergence of infested brood results in natural highest mite mortality during March and November. Two small peaks from infested brood were observed in June after pollen and nectar flow from *Acacia* and in November when colonies were placed in *Ziziphus* plantations during October 1994.

By linking this present periodic natural mite mortality data collected over 14 months a mite population model can be worked so that an accurate and simple method or tool for assessing the number of live mites within the colony is available for use by Pakistani beekeepers if they could monitor the debris samples.

CONCLUSION

This study reveals that T. clareae is an iteroparous parasite. showing continuous reproduction throughout the year in untreated colonies in the sub-tropical climate of Islamabad, having peaks and troughs through different seasons. The two seasons for higher reproductive ability being spring (February-March) and autumn (October-November). The two seasons of lower reproductive ability is cold winter (January) and humid late summer in (July). There are two peaks for natural mite mortality, Once in March and second time in November. The least mite mortality was observed in September.

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Table 1: Comparative seasonal population fluctuations of *T. clareae* in *A. mellifera*

Colonies in Group A and B

		Mites/debris			Mites/debris	
Period of	No of	Group A	S.E.	No of	Group B	S.E
Observat	Colonies	Low-	A	Colonies	Infested	B

ion	Examine d	infested Apistan Treated		Examined	No Treatment	
Oct-93	4	1.5	1.19	4	201	194
Nov-93	4	0.75	0.47	4	232	145
Dec-93	4	1.5	0.866	4	352	206
Jan-94	4	1.5	0.957	4	217	68.9
Feb-94	4	0.5	0.25	4	755	283
Mar-94	4	5.5	1.76	4	3584	1023
Apr-94	4	107	18	4	1955	437
May-94	4	215	94.7	4	332	167
Jun-94	4	1647	700	4	351	203
Jul-94	4	776	387	4	214	50.9
Aug-94	4	30	14.2	4	72	15.9
Sep-94	4	7	4.09	4	21	6.02
Oct-94	4	6	2.68	4	139	83.7
Nov-94	3	21	8.33	2	650	345
Dec-94	2	14	4.51	2	328	260

Table 1: Mean mite infestation in bimonthly brood and bee samples in apistan treated Group A

Month	Nov-93	Janury 94	Mar-94	May-94	Jul-94	Oct-94	Dec-94	Jan-95
in brood	0	0	0	8.3	0	0	0.3	0.25
S.D	0	0	0	7.44	0	0	0.61	0.5
On bees	0.12	0	0	1.31	0.19	0.09	0	0
S.D	0.24	0	0	1.52	0.38	0.19		0

Table 2: Mean infestation in bimonthly brood and bee samples in untreated Group B

Month	Nov-93	Jan-94	Mar-94	May-94	Jul-94	Oct-94	Dec-94	Jan-95
In brood	13.6	3.97	8.83	14.01	5.7	10.2	5.25	3
S.D	16.1	2.91	2.26	15.76	2.9	6.95	10.5	4.24

On bees	0.48	0.44	1.43	0.66	0.09	0.3	0.08
S.D	0.97	0.61	2.85	0.81	0.18	0.4	0.17

Table 3: Comparative seasonal relationship between mean percentage infestation of honeybee brood (B) and worker adults (W) by T.clareae (T) mites in Apistan treated low infested colonies in Group A at Islamabad during 1993-94.

Location	Season	Month	No of colonies	% brood cell	% worker	B/W
NARC	Winter	Nov-93	4	0	0.12	0
	Winter	Jan-94	4	0	0	0
	Spring	Mar-94	4	0	0	0
	Summer	May-94	4	8.26	1.31	6.3
	Summer	Jul-94	4	0	0.19	0
Chakwal	Autumn	Oct-94	4	0.3	0.09	0.03
NARC	Winter	Dec-94	3	0.25	0.09	2.77
	Winter	Jan-95	3	0.26	0	0

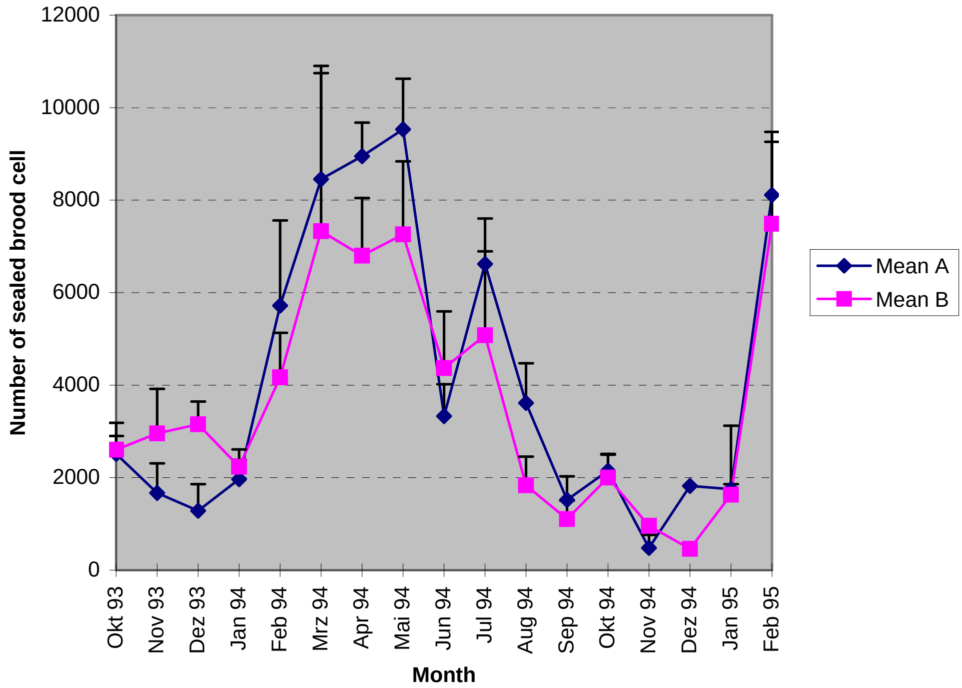
Table 4: Comparative seasonal relationship between mean percentage infestation of honeybee brood (B) and worker adults (W) by *T.clareae* (T) mites in untreated. Colonies in Group B at Islamabad during 1993-94

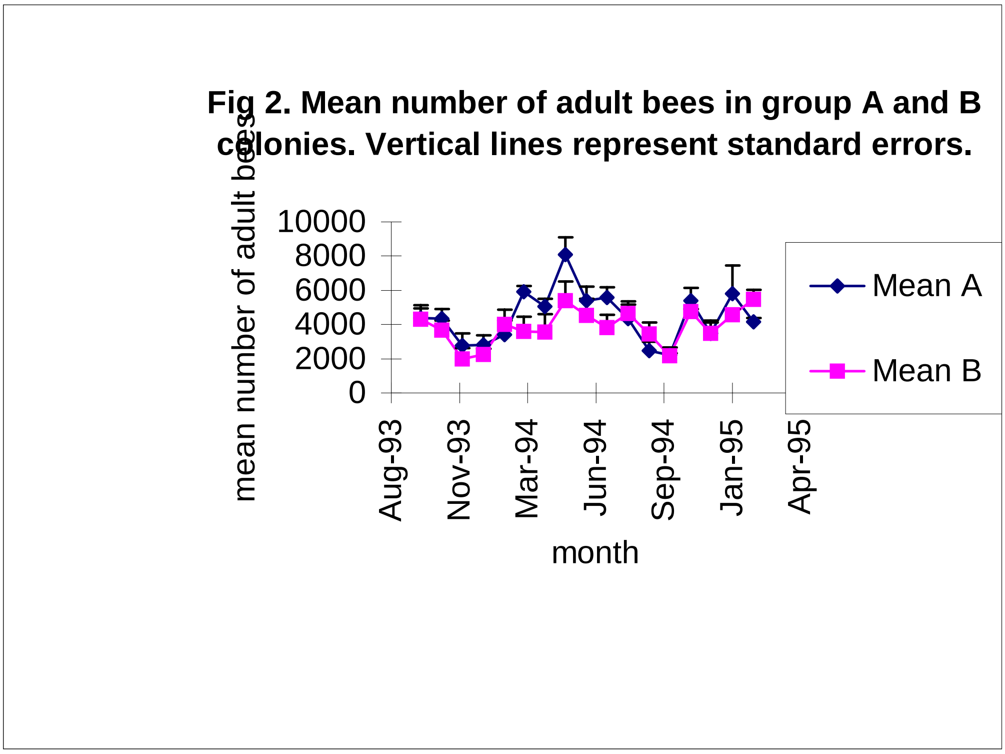
Location	Season	Month	No of colonies	% brood cell	% worker	B/W
Islamabad	Winter	Nov-93	4	13.6	0.48	28.33
NARC	Winter	Jan-94	4	3.97	0.44	9.02
	Spring	Mar-94	4	8.83	1.43	6.17
	Summer	May-94	4	14.01	0.66	21.22
	Summer	Jul-94	4	5.7	0.09	63.3
Chakwal	Autumn	Oct-94	4	10.2	0.3	34
NARC	Winter	Dec-94	2	5.25	0	5.25
	Winter	Jan-95	2	3	0.08	37.5

Table 5: Comparative annual honey yield in Group A and B

Month	Crop	Group A Treated	Group B Untreated
May-94	Acacia	20.65kg	10.23 kg
S.E		2.61	6.55
Jun-94	Clover	7.53 kg	5.69 kg
S.E		1.12	4.31
Oct-94	Ziziphus	7.9 kg	7.48 kg
S.E		3.1	2.06
Total	Annual	28.55	17.91
S.E		3.78	7.86

Fig 1. Mean number of sealed brood cell in group A and B
Vertical lines represent standard errors





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