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PARASITE-HOST INTERRELATIONSHIPS OF *BLASTOCRITHIDIA TRIATOMAE* AND TRIATOMINES

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In first clinical trials with the nitrofuran Lampit® (BAYER AG), parasitological cure of Chagas' disease was demonstrated by xenodiagnosis. However, different results were obtained using *Triatoma infestans* from two insectaria (Haberkorn, personal communication); careful examination of the insects demonstrated that some bugs from one insectarium possessed an infection with an unknown homoxenous trypanosomatid, *Blastocrithidia triatomae* (Cerisola et al., 1971).

The limited observations so far made indicate that *B. triatomae* is influenced by conditions in the bug. Blood ingestion of the bug can be deleterious for the stomach population of *B. triatomae*. Like *T. cruzi*, *B. triatomae* is lysed by blood from chickens and rats but not by blood from mice (Schaub, 1988; Schaub & Dages, 1985). In addition, more drought-resistant cyst stages develop in the rectum, with increasing percentages during periods of starvation of the bug (Schaub & Reduth, unpubl.).

More information is available about influences of *B. triatomae* on the insect. Haberkorn (1976) first observed pathological effects of *B. triatomae* on *T. infestans*. Realizing that this offered a possibility for biological control of vectors of Chagas' disease I started detailed investigations. The results of 11 years of work are just now being published in extensive

form. Most of our studies were performed with *T. infestans*, but effects in other triatomines were similar.

In our early studies, infection was achieved naturally by rearing infected older instars and uninfected first instars (L1) together. Bugs were fed weekly on chickens. The parasite was transmitted between bugs by coprophagy and/or cannibalism (Schaub et al., submitted). Several pathological effects of *B. triatomae* were observed: Blood-red faeces containing undigested haemoglobin were deposited regularly. The small intestine was often dilated and by transmission electron microscopy absence of extracellular membrane layers or microvillar borders in this region of the intestine could often be demonstrated. Sometimes gut-wall cells were totally destroyed so that the basal lamina was accessible to parasites (Jensen, 1987). Such damage might lead to the light red colour of the haemolymph which we very rarely observed. However, a flagellate inside the gut cells was never seen, only some flagella which were pushed into the cells.

This behaviour of flagellates was also observed in the upper region of the Malpighian tubules. In addition, the number and size of concretions in the cells of Malpighian tubules of infected bugs increased by about 100% as compared to uninfected bugs. In some parts of the Malpighian tubules the microvillar border was reduced (Schaub & Schnitker, submitted). During the first 4 and 24 hours after feeding infected fifth instars excreted approximately a 2.5-fold smaller volume of urine. Surprisingly, the *in vitro* secretion rates of Malpighian tubules from these bugs and from uninfected bugs were nearly identical. Storage and release of diuretic hormone in infected bugs induced normal *in vitro*

secretion rates. Therefore, reductions of the tracheal system in infected bugs might be responsible for the discrepancy between *in vivo* and *in vitro* excretion (Schnitker et al., 1988).

In addition to disturbed digestion and excretion, tanning was regularly retarded and/or reduced. Occasionally larvae and adults with a yellow cuticle, like that of uninfected bugs shortly after ecdysis were observed, but this colour remained unchanged for more than 2 weeks. Haemolymph amino-acid analysis revealed that the concentration of tyrosine, which is used for sclerotization, was significantly reduced in infected bugs (Schaub & Schmidt, unpubl.).

In quantitative studies on the development of groups of L1s reared with uninfected or infected older instars the first moults in all groups occurred at the same time, but in the majority of groups with infected *T. infestans*, larval development was strongly retarded in late instars. 16 weeks after first feeding of L1s 50% of uninfected L5s had moulted to the adult stage, whereas the same proportion of infected bugs needed 22 weeks. In addition to increased developmental times, mortality rates in groups exposed to infection were higher than in uninfected groups (Table I).

Such studies in which the bugs could infect themselves coprophagically were performed with different strains of *T. infestans* and different Triatominae. Retardation of development was generally observed in the last instars, except with the three *Rhodnius* species (Schaub, 1988 a; Schaub & Breger, in press; Schaub, unpubl.). Mortality rates seem to be correlated with total infection rates of groups (dead larvae plus surviving adults) (Table I).

TABLE I: Mortality rates in uninfected groups and mortality and infection rates in groups exposed to *B. triatomae* (mean and SD as percentages)

species	uninfected groups	*B. triatomae*-groups	
	mortality	mortality	infection rates
T. infestans	10 ± 4	85 ± 10	93 ± 6
T. sordida	22 ± 14	41 ± 20	49 ± 27
T. pallidipennis	46 ± 9	59 ± 10	11 ± 7
T. spinolai	40 ± 18	85 ± 13	69 ± 16
D. maxima	24 ± 10	94 ± 7	48 ± 15
R. prolixus	25 ± 3	16 ± 15	34 ± 6
R. robustus	22 ± 17	11 ± 4	5 ± 7
R. neglectus	14 ± 3	17 ± 11	2 ± 3

A correlation of infection rate and mortality rate could clearly be demonstrated by adding different numbers of infected older larvae to the uninfected L1s of *T. infestans*: Addition of more infected bugs resulted in higher infection rates and, thereby, higher mortality rates. If infected and uninfected bugs were added, infection rates and pathological effects were similar to groups with the same number of infected older larvae. Therefore, bugs did not discriminate between faeces from infected and uninfected bugs, or else they did not reject infectious faeces (Jensen, 1984). Dry faeces had to be redissolved by fresh faeces before infection was possible (Schaub et al., submitted).

In these experiments the exact time of infection and the infection dose could neither be influenced nor determined. Therefore, methods for isolation of drought-resistant cyst stages of *B. triatomae* (Schaub et al.,

in press) and for feeding a cyst/blood mixture were developed. Afterwards, influence of experimental design on the pathological effects of *B. triatomae* were evaluated, especially with *T. infestans*.

A first series of experiments with L1s reared singly and in groups of 20, 30, 40, and 50 larvae showed that development of some larvae is apparently retarded by isolated rearing (not caused by lack of symbionts). This effect could be observed in uninfected and infected bugs. In uninfected groups developmental time was not influenced by group size. In contrast, experimentally infected groups of 50 larvae showed a stronger developmental retardation and a statistically significant higher mortality rate than the other infected groups (Student's t-test: $p<0.05$), indicating a crowding stress (Schaub, unpubl.).

Since natural populations cannot suck blood regularly, we studied the influence of starvation on the pathological effects of *B. triatomae*. Increase of mortality rate was similar in bugs with weekly feeding intervals and in those starved for 2-3 months in each instar (Schaub, unpubl.).

In a third series of experiments infection doses were varied between 10^4 and 10^8 cysts/ml blood. Thus, each L1 of *T. infestans* ingested 80 to 800,000 cysts. All L1 became infected, but pathological effects in the groups with different infection doses were similar and occurred in the last instars (Schaub & Rohr, unpubl.). This was presumably caused by the fact that *B. triatomae* excystates in the small intestine and not in the stomach (Schaub & Pretsch, 1981) and that only small portions of the ingested blood were given to the small intestine at a time. High division rates of *B. triato-*

mae resulted in a similar level of parasites 4 weeks after infection with 10^4 or 10^8 cysts/ml.

Variation of maintenance temperature also showed that *B. triatomae* needed some time before effects occurred. Normally maintenance was at 26° C; lower temperatures retarded the development of *T. infestans* and increased the pathological effects of *B. triatomae*. Maintenance at higher temperatures shortened the developmental times, and more bugs reached the adult stage. Variations of relative humidity did not influence the pathological effects (Schaub, unpubl.).

Infections of different instars also demonstrated the time-lag before pathological effects were observed. After infection of L1s, L2s, L3s or L4s the first developmental retardations were evident in the moults of the L3s, L4s, L5s and L5s, respectively, but were nearly undetectable after infection of L5s. Mortality rates of fifth instars also reflect this effect: 58%, 38%, 26%, 25% and 5% of L5s died after infection of the respective instar (Schaub & Wolf, unpubl.).

Development of the resulting adults was also strongly affected; 50% of the infected females or males were dead after 9 or 12 weeks, respectively. Individual infected females lived up to 20 weeks and males up to 27 weeks. In uninfected males the mean life times were 35 weeks after moult to the adult stage and 30 weeks in females. Reproduction of uninfected adults was influenced by blood ingestion and aging. However, in infected groups or pairs the number of laid eggs/day, egg weight, hatching rate, and weight of the L1s was always reduced as compared to controls (Schaub et al., 1984).

In a further series of experiments we infected L1s of *T. infestans*, fed them last in the second, third or

fourth instar and investigated the effects of *B. triatomae* on starvation capacity. Infection with *B. triatomae* reduced life spans by 51%, 55%, and 32%, respectively (Schaub & Lösch, submitted).

These detailed studies with *T. infestans* gave further information about the pathogenic mechanisms. More importantly, they showed that the pathological effects in our previous studies were not caused by the experimental conditions, especially group size or feeding intervals. Therefore, studies of different species could be performed using an infection dose of about 10^3 cyst stages/L1. The pathological effects on developmental times were similar to those obtained after coprophagic infection, but mortality rates were higher because of an infection rate of 100% (Table II).

TABLE II: Mortality rates in uninfected and *B. triatomae*-infected groups (mean and SD as percentages)

species	uninfected groups	*B. triatomae*-groups
T. infestans	10 ± 10	85 ± 4
T. sordida	20 ± 23	86 ± 12
T. pallidipennis	44 ± 6	66 ± 8
T. spinolai	40 ± 18	82 ± 15
D. maxima	17 ± 7	97 ± 3
R. prolixus	25 ± 16	14 ± 6
R. robustus	22 ± 17	18 ± 14
R. neglectus	14 ± 3	17 ± 6

High mortality rates of uninfected *T. pallidipennis* and *T. spinolai* indicated additional stress factors besides *B. triatomae*. (Great difficulties with the colony

of *T. spinolai* occurred one generation later, in the fourth generation.) Preliminary results show that *T. braziliensis* and *P. megistus* were also affected by *B. triatomae* (Schaub, unpubl.). In agreement with the experiments with coprophagic infection, the three *Rhodnius*-species reacted tolerantly. At first we suspected that their rapid normal development might have been responsible. However, increasing the developmental time by longer starvation periods did not change the results with *R. prolixus*, nor did the use of sterile L1s (whereby an early uptake of symbionts from infected older larvae was achieved). These results indicate that species of the genus *Rhodnius* tolerate infections with *B. triatomae*.

It is an interesting phenomenon that pathogenesis in *T. rangeli*-infected *R. prolixus* is very similar to that in *B. triatomae*-infected *Triatoma* species. In addition, *T. rangeli* affects *R. prolixus* and *R. robustus*, but not *T. infestans* (Anez, 1984; D'Alessandro, 1976), just the opposite situation of influence as with *B. triatomae*. According to a theory of Watkins (1971), the pathological effects of *T. rangeli* might result from a direct action on the bug's symbionts.

Since symbionts of triatomines should supply the bug with B-vitamins, we studied the effect of vitamin-B-complex supplemented sheep blood on the development of *B. triatomae* in *T. infestans* and on the development of infected bugs (Jensen, 1987). Supplement of B-vitamins resulted in significantly more *B. triatomae* in the small intestine of young instars; later no such effect was obtained. The pathological effects of *B. triatomae* on developmental time and mortality were strongly reduced, supporting the theory that *B. triatomae* affects

the vitamin supply of the bugs. A possible difference in the number of symbionts in uninfected and *B. triatomae*-infected bugs is now being investigated.

Apart from these pathological effects on vectors of Chagas' disease, competition of *B. triatomae* and *T. cruzi* should be considered in double infections. Both flagellates colonize the whole intestinal tract and the Malpighian tubules, preferring the rectal pads in the rectum (Böker & Schaub, 1984; Schaub & Böker, 1986 a; b). Quantitative light microscopical studies indicate a suppression of *T. cruzi* in double infections; nonetheless *T. cruzi* was not eliminated during the first 20 weeks p.i. and metacyclic trypomastigotes always developed (Schaub & Mehl, in preparation). Since morphological classification of both flagellates might include incorrect identifications, an additional marker is needed. Lectin gold labelling has been successfully tested for transmission electron microscopy (Zimmermann et al., 1987) and can also be used for investigations of early colonization behaviour in the scanning electron microscope.

Summarizing our investigations, *B. triatomae* is influenced by blood ingestion and starvation of the host. More important is the pathogenity of *B. triatomae* for triatomines and the probable suppression of *T. cruzi* in double infections. All our data point to the potential of using *B. triatomae* as a biological agent against the main vectors of Chagas' disease. Field trials should first of all clarify how high infection rates could be achieved.

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