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Serratia marcescens as a pathogen of tsetse flies

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Summary

When applied to the ears of rabbits used as hosts for tsetse flies, the bacterium *Serratia marcescens* produced significant mortality in populations of *Glossina m. morsitans* and *G. pallidipes.* After being ingested during the blood meal, cells of *S. marcescens* multiplied in the intestine of the flies and entered the hemocoel. Using the brush method of applying the bacterium, 100% mortality of both *Glossina* species occurred within 10 days after application. In newly killed flies, the bacteria could be found free in the hemocoel as well as in the fat body and blood cells. The supernatant of a liquid culture of *S. marcescens* did not produce fly mortality when applied to rabbit ears. The results indicate that *S. marcescens* is able to invade the hemocoel of "normal" laboratory-reared tsetse flies.

Key words: Glossina; tsetse flies; *Serratia marcescens;* insect pathogen.

Introduction

In spite of its persistent character and the numerous investigations conducted on its biology, few pathogens of tsetse flies have been found. A preliminary investigation made in Amsterdam (Poinar et al., 1977) demonstrating the effect of some commonly-known insect pathogens *Glossina m. morsitans* showed that a strain of the bacterium, *Serratia marcescens* was pathogenic to adult flies.

Even before the thorough study of Steinhaus (1959), *S. marcescens* had been recovered from dead or dying insects and was regarded as a possible general insect pathogen. Easily identified by its red color (white strains commonly occur), close-coiled peritrichous flagella and biochemical characters (listed in Poinar and Thomas, 1978), the bacterium has been considered a faculta-

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tive pathogen lacking the ability to invade the hemocoel of normal, healthy insects (Bucher, 1963). In nature, only members of the Acrididae have been found infected with *S. marcescens* and there is no report of a higher dipteran succumbing to this bacterium.

The present investigation was undertaken to determine if *S. marcescens* actually could kill "healthy" tsetse flies and if so, how the infection occurred.

Materials and methods

The colony of *G. m. morsitans* Westw. was maintained at the University of Amsterdam since 1973, having originated from the Binga and Kariba districts in Rhodesia. The colony of *G. pallidipes* Austen was received in 1975 from Mrs. E. A. Opiyo, then working at the East African Trypanosomiasis Research Organization at Tororo, Uganda. Both colonies were fed on the ears of rabbits five times per week.

The strain of *S. marcescens* Bizio used here came from the culture collection of the Invertebrate Pathology Diagnostic Laboratory at the University of California, Berkeley and was designated as strain 0-41-1. It was originally isolated from the pyralid, *Chilotraea infuscatella* in Taiwan. The bacteria were grown on nutrient agar plates maintained at 30° C. Inocula were obtained by flooding the plates with sterile water and then either applying the solution with a 5 cm wide paint brush or a garden hand sprayer. A total of 30 male and female *G. morsitans* and *G. pallidipes*, respectively, were placed on rabbits whose ears had been brushed with the bacterial suspension. No attempt was made to estimate the number of bacteria applied to the surface using this method. A total of 20 male and female of *G. morsitans* and *G. pallidipes*, respectively, were placed on rabbits whose ears had been sprayed with the bacterial suspension. In this experiment, 4 ml of the suspension were sprayed on each ear and it was estimated with the plate dilution method that each ml of inoculum contained approximately 10^7 cells of *S. marcescens*.

Treated and control flies were placed on the rabbits' ears for 30 min or until all flies had fed, then held in environmentally controlled chambers at 25° C and 80% relative humidity.

In order to test for a possible toxin, cells of *S. marcescens* were grown in a solution consisting of 3 g yeast extract, 5 g peptone and 3 g NaCl in a liter of water: After 72 h, the suspension was centrifuged and the remaining supernatant separated into 2 portions, one of which was heated at 80° C for 5 min.

Approximately 4 ml of each portion were brushed on the ear of a rabbit that immediately afterwards served as host for 20 adults of *G. morsitans* and *G. pallidipes*, respectively.

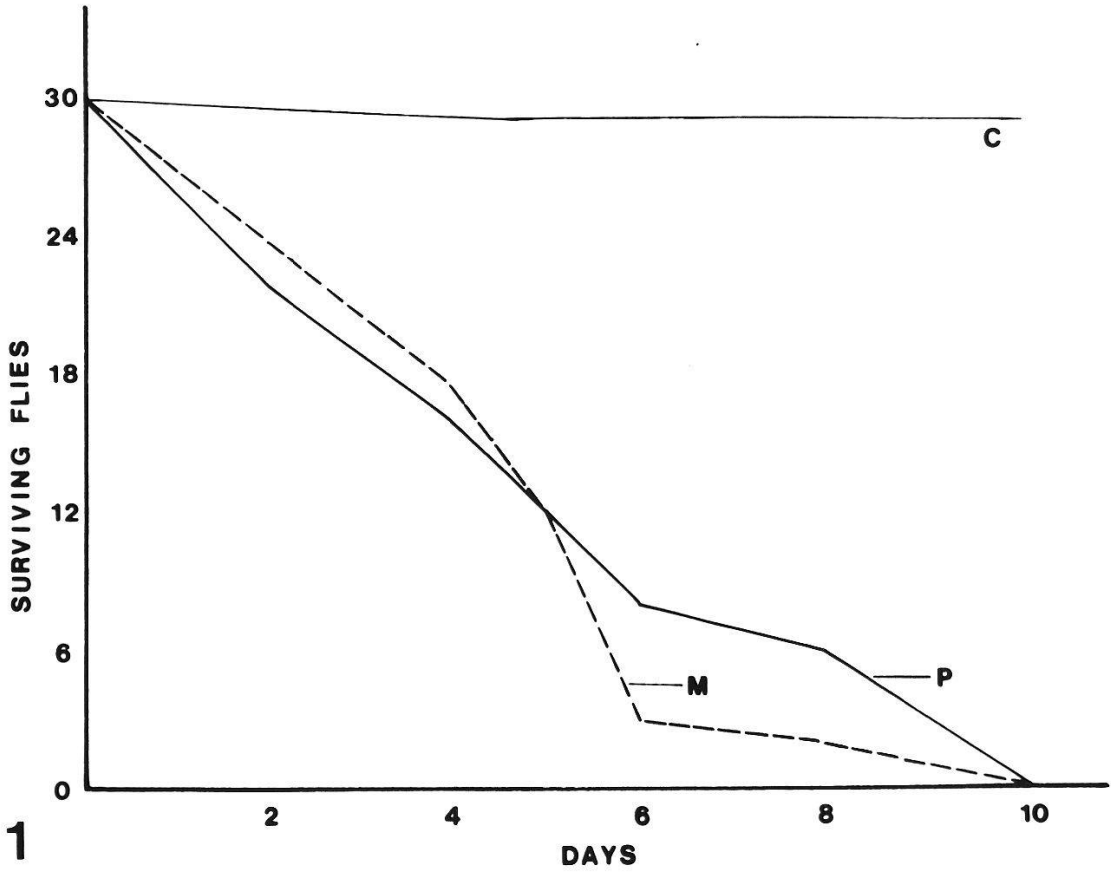
All flies were examined daily and dead ones were dissected and a drop of hemolymph or piece of tissue removed and placed on nutrient agar.

Results

With the brush method of application, all 60 adults of *G. morsitans* and *G. pallidipes* died as a result of septicemia within 10 days after treatment (Fig. 1). With the spray technique, 15 and 18 flies (out of a total of 40) of *G. pallidipes* and *G. morsitans*, respectively, died from septicemia within 10 days after treatment (Fig. 2). All dead flies from the treated ears contained cells of

Fig. 1. Mortality of *Glossina m. morsitans* (M) and *G. pallidipes* (P) after feeding on rabbit ears brushed with a suspension of *Serratia marcescens*. C = control.

Fig. 2. Mortality of *Glossina m. morsitans* (M) and *G. pallidipes* (P) after feeding on rabbit ears sprayed with a suspension of *Serratia marcescens*. C = control.


30
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SURVIVING FLIES
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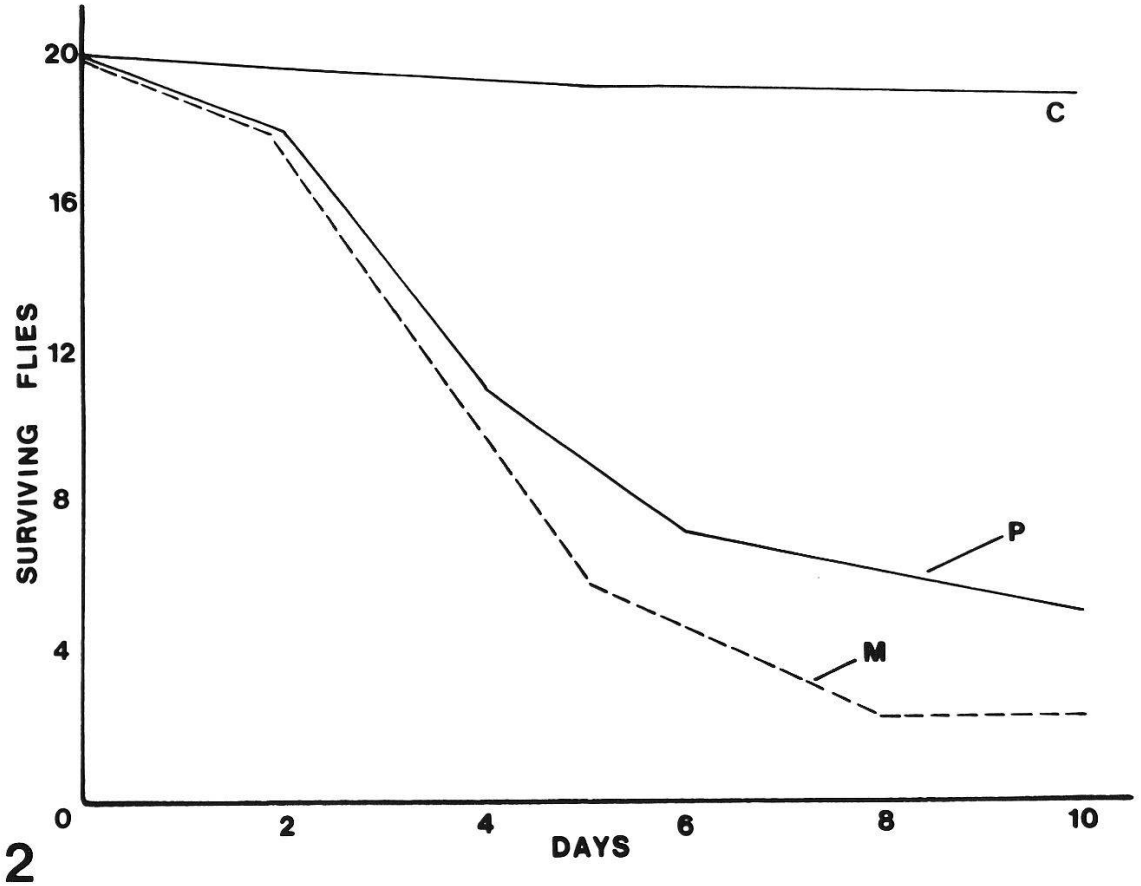
DAYS

1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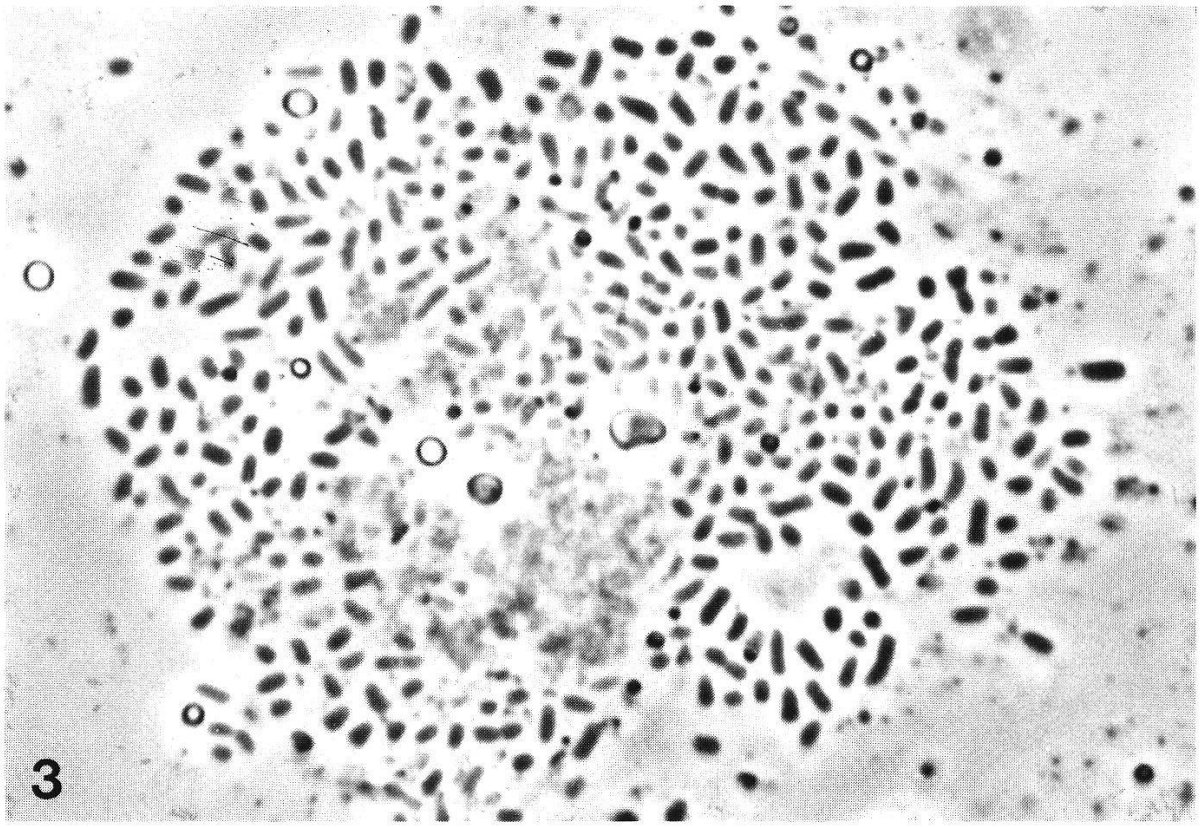

Fig. 3. Cells of *Serratia marcescens* within a hemocyte from a recently killed tsetse fly (× 1200).

S. marcescens in their hemocoel at the time of death. The rods could be found in the hemolymph, or within the fat body or blood cells of the newly-killed flies (Fig. 3).

No mortality resulted when normal or heated bacterial supernatant was applied to the rabbits' ears.

An examination of the intestinal contents of flies dying from *S. marcescens* showed high bacterial populations in the gut lumen.

The few control flies that died during the experimental period lacked cells of *S. marcescens* in their hemocoel. No ill effect of the treated rabbit was noticed during or after the experiments.

Discussion

Cells of *S. marcescens* ingested by adult *G. morsitans* and *G. pallidipes*, respectively, multiplied in the intestine and invaded the hemocoel of a large percentage of flies, resulting in septicemia and mortality.

Up until now, as Goodwin (1968) indicated, there has been no direct evidence that primary invasive mechanisms of *S. marcescens* existed. Thus, the present study is interesting from two aspects. It shows that, at least in adult tsetse flies, this particular strain of *S. marcescens* is able to multiply inside the intestinal lumen and actually traverse the gut wall and enter the hemocoel of

"normal" flies. Probably a certain number of cells must be ingested before the colonies become established and this is why the aerial suspension method of application was less effective than the brush method, assuming that less bacteria were applied with the former method. Then again, a certain bacterial population probably must be reached in the gut before invasion into the hemocoel is possible and this is why the flies died over a period of several days.

Although the supernatant trials were negative, it is still possible that invasion into the hemocoel is accomplished after the gut cells are damaged by a toxin or exoenzyme produced by the bacteria. Faust (1974) considered that toxins or enzymes were probably the cause of pathogenicity with *S. marcescens.*

The other interesting aspect of this study is the indication that the intestine of *Glossina* is very sensitive to the action of *S. marcescens* and possibly other bacteria. Briggs et al. (1977) discussed earlier reports of bacteria associated with tsetse flies and mentioned two bacteria, e.g. *Bacterium mathisi* and *B. prodigiosus* (this reference probably concerned *Serratia marcescens*) that were acquired through feeding and caused mortality of adults. Unfortunately, there are no remaining isolates of the above two species and they cannot be tested under more controlled conditions.

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