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## Scientific note

### A scientific note on in vitro experimental infection of American foulbrood in honeybees *Apis mellifera* L.

Tomoko Sakogawa\*, Shigeaki Kataoka, Atsuko Okayama,  
Chie Nakajima, Toyoaki Hayama

Research Institute for Animal Science in Biochemistry and Toxicology, 3-7-11 Hashimoto-dai,  
Sagamihara, Kanagawa, 229-1132 Japan

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#### *Apis mellifera* / American foulbrood / *Paenibacillus larvae* / in vitro infection

The in vitro experimental infection system of honeybee American foulbrood (AFB), which was also reported by Peng et al. [1, 2], was tested. The in vitro method enables a close observation of the disease process during the larval stage, because dead and possibly moribund larvae are removed rapidly from the cells by worker bees in the field hives. Since reproductibility and a high degree of larval survivorships in non-infected group were essential for our purpose, the method used was simplified as much as possible and used conventional aseptic procedure. The larval food used corresponded roughly to a 1.5 fold dilution of raw royal jelly with isotonic sucrose solution, because queen larvae were also susceptible to AFB.

One-day-old larvae (the first instar) of honeybees in the field hives were transferred onto the food containing spores of *Paenibacillus larvae*, the causative agent of AFB, in a small culture dish, and fed for 24 h. The dish was kept at 34 °C and 98 % relative humidity in an incubator. The number (colony forming unit) of spores in food of each group was 0 (control)  $10^2$ ,  $10^3$  or  $10^4$  spores per mL. The larvae were transferred every day onto the fresh food without spores in a new dish until the beginning of the pupal stage.

Mortalities of larvae by 4th and 7th days and isolation rates of *P. larvae* in dead larvae increased in relation to the number of spores in

the food. A slightly lower susceptibility of 2-day-old larvae and no susceptibility of 3-day-old larvae to the infection were confirmed. Contaminations by bacteria other than *P. larvae* were negligible in 1-day-old groups but increased with age when larvae were collected from the combs in field colonies.

The in vitro infection system can be applicable to further studies on pathogenesis of AFB and drug efficacy.

#### Note scientifique sur l'infection expérimentale in vitro de la loque américaine dans des colonies d'*Apis mellifera* L.

#### Wissenschaftliche Notiz über die experimentelle in vitro Infizierung von Honigbienen mit Amerikanischer Faulbrut.

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\* Correspondence and reprints  
E-mail: tikuan@pa2.so-net.ne.jp