

Bacillus thuringiensis subsp. *konkukian* (Serotype H34) Superinfection: Case Report and Experimental Evidence of Pathogenicity in Immunosuppressed Mice

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We present a case of severe war wounds infected by *Bacillus thuringiensis* serotype H34 and describe the experimental protocol used to demonstrate its ability to infect mice after cutaneous inoculation. This case is interesting because *B. thuringiensis* is considered to be a contaminant in laboratories and receives inadequate attention.

Bacillus species are widely distributed in nature, and finding a *Bacillus* sp. in medical practice, aside from *B. anthracis* and *B. cereus*, is often considered clinically irrelevant. *B. thuringiensis* is closely related to *B. cereus* and is used extensively around the world as a pesticide in forestry and agriculture (1). The two species can be differentiated only by the production of the plasmid-encoded delta endotoxin, which is pathogenic for larvae of *Lepidoptera*. As this characteristic is not consistent, many consider *B. thuringiensis* to be a variant of *B. cereus*.

Infection in humans is unusual and, except for reports on infection of the gastrointestinal tract or laboratory contamination, there is only one clinical report of infection by this microorganism (2). We describe a case of severe war wounds infected by *B. thuringiensis* serotype H34 (4) and present experimental evidence for the pathogenicity of this strain in a mouse model of cutaneous infection.

Case report. The patient was a healthy 28-year-old French soldier severely wounded in March of 1995 by a land mine explosion in former Yugoslavia. When he was admitted to the emergency room of the French military hospital in Sarajevo, Yugoslavia, the patient presented with hemorrhagic shock, a pulmonary blast, shrapnel lesions in the left leg, and multiple fractures in the left knee. Immediate antibioprophyllaxis, given on the battlefield, included penicillin G (5×10^6 U/24 h) associated with metronidazole (1.5 g/24 h). After immediate surgery, the patient was evacuated within 24 h to the Begin Army Hospital (Paris, France). Upon admission, he was found to have an oral temperature of 38.7°C, a pulse of 100 beats/min, blood pressure of 130/90 mm Hg, and a respiratory rate of 24 breaths/min. His leukocyte count was 10,200 cells/mm³. He was immunocompetent and a nonsmoker and had no history of illness.

Physical examination revealed abscesses on the left thigh and knee, and biopsy specimens of the wounds were obtained surgically. Microscopic examination showed large gram-positive rods with endospores. After routine culture, the colonies on blood agar medium were shown to be large (5 mm), beta-hemolytic, flat, white, and rough. Catalase was positive, and

oxidase was negative. The bacterium was mobile. Biochemical tests performed with API 50-CHB and 20-E (Biomerieux, S. A., Lyon, France) identified the strain as *B. cereus*, disagreeing with the results obtained by the Vitek system (Biomerieux Vitek, St. Louis, Mo.), which identified the strain as *B. thuringiensis*. The results of the biochemical reactions are presented in Table 1.

As this finding was unusual, the strain was sent to M. Lecadet and her colleagues at the World Health Organization collaborating center for entomopathogens (Unité des Bactéries Entomopathogènes, Institut Pasteur, Paris, France), who identified the strain as *B. thuringiensis* subsp. *konkukian* (serotype H34).

The presence of crystal in sporulated culture, which is characteristic of *B. thuringiensis*, was recorded by direct examination of a fresh preparation under a phase-contrast microscope or after specific coloration of proteins with Coomassie brilliant blue solution.

The standard characteristics according to the method described by Sneath (5) were determined mainly by the API *Bacillus* (API-20E and API-50CH) system.

H serotyping based on flagellar antigens was performed according to the agglutination method described by de Barjac (3) with specific antisera for the 79 known *B. thuringiensis* serovars. In brief, an early exponentially growing culture is inoculated on the agar surface of a small, special tube filled with soft nutrient agar. If mobile, the bacteria migrate in 24 h. The cells obtained by this method are then tested for agglutination with various antisera.

Antimicrobial susceptibility was tested by E test and disk diffusion. Interpretive criteria for *Staphylococci* were used because there are no National Committee for Clinical Laboratory Standards guidelines for interpreting the results of susceptibility tests on *Bacillus* spp. Results (MICs in micrograms per milliliter and zone diameters in millimeters) showed resistance to penicillin G (MIC, 32; diameter, 8) and ampicillin (MIC, 16; diameter, 12) and a paradoxical susceptibility to piperacillin (MIC, 1; diameter, 27). The presence of β -lactamase was indicated by the Cefinase test (Biomerieux), performed in liquid medium. The strain was also susceptible to imipenem (MIC, 0.047; diameter, 34 mm), vancomycin (MIC, 2; diameter, 18), gentamicin (MIC, 0.5; diameter, 24) and ciprofloxacin (MIC, 0.25; diameter, 31).

The patient recovered after 15 days of intensive care, multiple surgical cleansings, and an antibiotic treatment of cipro-

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TABLE 1. Biochemical reactions of *B. thuringiensis* serotype H34 isolated from war wounds

Test material or process	Result
Catalase	Positive
Oxidase	Negative
MacConkey agar.....	No growth
Trypticase soy agar.....	Growth
Potassium thiocyanate.....	Growth
Blood agar.....	Beta-hemolysis
Thermophilia.....	Negative
Voges-Proskauer.....	Positive
Nitrate reduction.....	Positive
Esculin hydrolysis.....	Positive
Lecithinase.....	Positive
Gelatin.....	Negative
Urea.....	Negative
Indole.....	Negative
Simmon citrate.....	Negative
Sucrose.....	Negative
Inulin.....	Negative
Ribose.....	Negative
Amygdalin.....	Negative
Trehalose.....	Acid
Xylose.....	Negative
Maltose.....	Acid
Galactose.....	Negative
Glucose.....	Acid
Salicin.....	Negative

floxacin associated with gentamicin that was administered for 10 days.

The isolation of *B. thuringiensis* from wound abscesses raised the question of whether this bacterium was the causative agent of the infection or simply a contaminant, leading us to investigate the potential pathogenicity of this strain in mammals with a mouse model of cutaneous infection. After depilation of a 2-cm² area of their skin, six groups of 5-week-old female BALB/c mice were infected by an application of a bacterial suspension containing, respectively, 10⁵, 10⁶, or 10⁷ CFU per mouse. For each inoculum, 4 days before the experiment, one group of mice was immunosuppressed by intravenous injection of cyclophosphamide (200 mg/kg of body weight); a control group was not immunosuppressed. Cutaneous inflammatory lesions occurred in all animals when the 10⁷ CFU inoculum was used. Lesions healed spontaneously after 48 h in nonimmunosuppressed mice but increased in the other group. Two days after the infection, the mice were sacrificed, and the inner side of the skin (4 cm²) was aseptically removed and grown on a blood agar medium. *B. thuringiensis* was recovered only in the samples from the immunosuppressed mice. The bacterial count was correlated with the quantity of the cutaneous bacterial inoculum. Bacteriological examination of tissue stained by Gram stain and periodic acid-Schiff stain revealed that *B. thuringiensis* was associated with tissue necrosis and polymorphonuclear infiltrates (Fig. 1).

The animal model used demonstrates the ability of *B. thuringiensis* subsp. *konkukian* (serotype H34) to produce infection and myonecrosis in the absence of an effective immune response. Likewise, the clinical data suggest that disease may also be produced in patients with tissue devitalization caused by massive tissue destruction. Like *B. cereus*, this bacterium forms spores which are normally found in the soil. Presumably, soil and dust particles projected by the land mine explosion were the source of the organism in this unusual case of infection. This reported episode of soft-tissue infection and necrosis by *B. thuringiensis* is reminiscent of *B. cereus* infection after severe trauma (6).

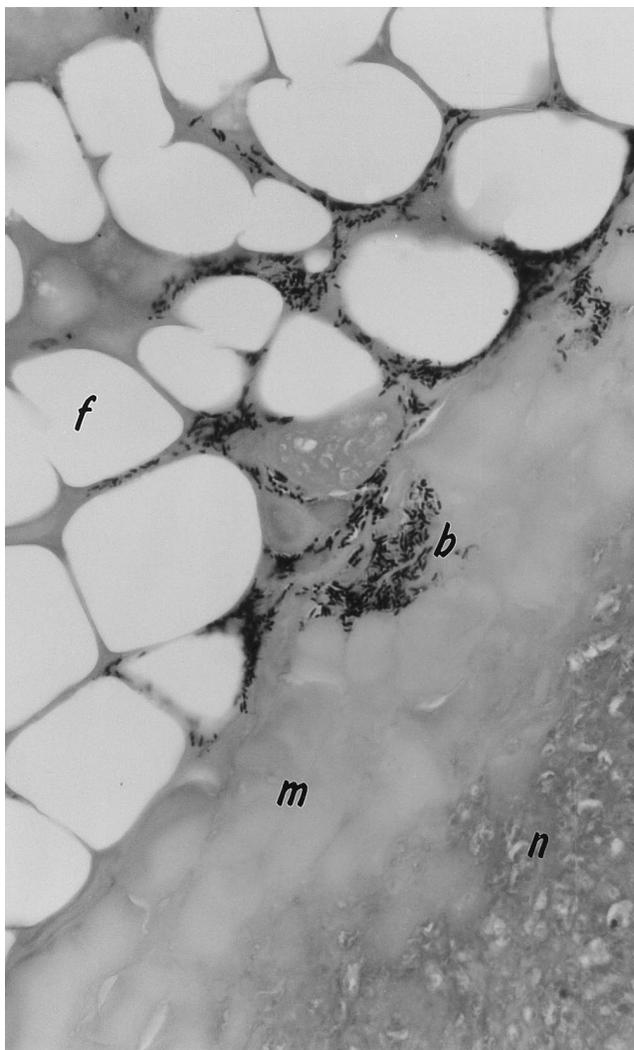


FIG. 1. Gram stain of tissue revealing association of *B. thuringiensis* with tissue necrosis and polymorphonuclear infiltrates. Abbreviations: b, bacteria; f, fat; m, muscle; n, deep necrosis. Magnification, $\times 400$.

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REFERENCES

- Aronson, A. I., W. Beckman, and P. Dunn. 1986. *Bacillus thuringiensis* and related insect pathogens. *Microbiol. Rev.* **50**:1-24.
- Damgaard, P. H., P. E. Granum, J. Bresciani, M. V. Torregrossa, J. Eilenberg, and L. Valentino. 1997. Characterization of *Bacillus thuringiensis* isolated from infections in burn wounds. *FEMS Immunol. Med. Microbiol.* **18**:47-53.
- de Barjac, H. 1981. Identification of H-serotypes of *Bacillus thuringiensis*, p. 35-43. In H. D. Burgess (ed.), *Microbial control of pests and plant diseases*. The Academic Press Co., London, England.
- Lee, H. H., J. A. Lee, K. Y. Lee, J. D. Chung, H. De Barjac, J. F. Charles, V. Cosmao-Dumanoir, and E. Frachon. 1994. New serovars of *Bacillus thuringiensis*: *Bacillus thuringiensis* ser. *coreanensis* (serotype H25), *Bacillus thuringiensis* ser. *leisis* (serotype H33) and *Bacillus thuringiensis* ser. *konkukian* (ser. H34). *J. Invertebr. Pathol.* **63**:217-219.
- Sneath, P. H. A. 1986. Endospore-forming Gram-positive rods and cocci, p. 1104-1140. In J. P. Butler (ed.), *Bergey's manual of systematic bacteriology*, 1st ed. Williams & Wilkins, Baltimore, Md.
- Wong, M., and M. J. Dolan. 1992. Significant infections due to *Bacillus* species following abrasions associated with motor vehicle related trauma. *Clin. Infect. Dis.* **15**:855-857.