Public Health Implications of the Microbial Pesticide *Bacillus thuringiensis*: An Epidemiological Study, Oregon, 1985–86

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Abstract: *Bacillus thuringiensis* var. *kurstaki* (B.t.-k) is a microbial pesticide which has been widely used for over 30 years. Its safety for a human population living in sprayed areas has never been tested. Surveillance for human infections caused by B.t.-k among Lane County, Oregon residents was conducted during two seasons of aerial B.t.-k spraying for gypsy moth control. *Bacillus* isolates from cultures obtained for routine clinical purposes were tested for presence of *Bacillus thuringiensis* (B.t.). Detailed clinical information was obtained for all B.t.-positive patients. About 80,000 people lived in the first year's spray area, and 40,000 in the second year's area. A total of 55 B.t.-positive cultures were identified. The cultures had been taken from 18 different body sites or fluids. Fifty-two (95 percent) of the B.t. isolates were assessed to be probable contaminants and not the cause of clinical illness. For three patients, B.t. could neither be ruled in nor out as a pathogen. Each of these three B.t.-positive patients had preexisting medical problems. The level of risk for B.t.-k and other existing or future microbial pesticides in immunocompromised hosts deserves further study. (*Am J Public Health* 1990; 80:848–852.)

Introduction

Bacillus thuringiensis var. *kurstaki* (B.t.-k) is a microbial pesticide which has been used for large scale pest eradication programs in populated areas for more than 30 years. Microbial pesticides are bacteria or viruses that are toxic or infectious to the target pest, usually an insect, but are considered to be harmless to mammals and most other non-target species. In the United States, these programs have been conducted primarily in the Northeast for control of lepidopterous pests, particularly gypsy moth, spruce budworm, and tussock moth infestations. Greater than one million pounds of this pesticide are applied annually in the United States alone.[1] In addition, the bacterium is widely used in commercial landscaping and is sold retail to gardeners for pest control.

Despite its wide use, particularly its aerial application over populated areas, B.t.-k has never been studied epidemiologically to assess its potential for causing human infection. Immunocompromised persons are probably the group most likely to be at risk of infection by a bacterium otherwise harmless to humans.

Bacillus thuringiensis (B.t.) is a gram-positive, spore-forming, facultative soil saprophyte, distinguished by the presence of a crystalline parasporal inclusion.[2,3] Because B.t.-k is not considered to be a mammalian pathogen it has been exempted from pre-harvest and post-harvest restrictions placed upon raw agricultural commodities.[4] Its cell size, 3.0–5.0 × 1.0–1.2 μm, makes it a respirable particle; the spores and toxin crystals of the commercial product are even smaller.[2]

Bacillus thuringiensis is distinguished from *Bacillus cereus* by the presence of a bipyramidal parasporal inclusion body or toxin crystal, which contains delta endotoxin. The toxin crystal is a plasmid-encoded protoxin that is activated under alkaline conditions such as are found in the gut of the gypsy moth larva. The mechanism of action of the pesticide in lepidopteran larvae is (Na,K)-ATPase inhibition, gut paralysis, gut necrosis and consequent cessation of feeding. In addition, the ingested spores may, after germination, result in larval septicemia.[2,5,6] The commercial formulations contain spores and toxin crystals, without any significant component of vegetative cells.

There is only one case of human disease associated with B.t.-k recorded in the medical literature. This occurred after a previously healthy 18-year-old farmer splashed a commercial product into his eye. He was treated with antibiotic ointment. Three days later, when the eye was still irritated, he was treated with a corticosteroid ointment. Ten days after the accident, a corneal ulcer was discovered and was successfully treated with subconjunctival injections of gentamicin and cefazolin sodium. *Bacillus thuringiensis* was cultured from the ulcer.[7,8]

Bacillus thuringiensis has been the subject of many animal and a few human experiments. In one study, 18 human subjects ingested one gram of a commercial B.t.-k product in capsules daily for five days. Five subjects also inhaled 100 mg of the powder daily for five days. No adverse health effects were noted on physical, laboratory, or roentgenologic examination. Laboratory examination did not include cultures of any site.[9]

Virtually all the animal experimentation has been done by agricultural laboratories. Most experiments have used rodents, but some assessed effects on non-target species of agricultural significance, such as farm animals and bees. Most have been done with small numbers of animals, usually without controls, and have shown B.t.-k to be harmless.[10,11]

In reviewing the *Bacillus thuringiensis* literature, distinctions must be made among the subtypes. *Bacillus thuringiensis* var. *israelensis*, for example, used for black fly and mosquito control, appears to be more toxic to mammals than B.t.-k, and has been implicated in a case of human cellulitis.[12] *Bacillus thuringiensis* var. *thuringiensis* has an exotoxin as well as the delta endotoxin, and has been cultured from multiple internal organs of animals autopsied after feeding studies.[10]

Some studies of B.t.-k have also shown pathogenicity of varying degrees. One experiment demonstrated that intraperitoneal injections of large doses of B.t.-k grown in culture caused death in seven out of 10 guinea pigs. Using *B.*

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cereus, the same procedure resulted in the death of five out of five guinea pigs. Needle trauma was ruled out as the cause of death, because none of five guinea pigs that were injected with *Bacillus subtilis* in the same experiment died.[9] B.t.-k and *B. cereus* were shown to persist in blood more than 48 but less than 72 hours following intraperitoneal infection[9]; inhalation, cutaneous injection, topical application to skin abrasions, and intragastric application all yielded no adverse effects in rodents.[9]

There is a single report in the agricultural literature of a fatal, naturally acquired, animal infection attributed to *Bacillus thuringiensis*, variety unknown. This was a case of bovine mastitis, presumably with secondary septicemia.[13]

Background of Present Study

In the summer of 1984, the Oregon Department of Agriculture discovered an infestation of gypsy moths in Lane County, located in the central portion of western Oregon and with a population of 260,000. The infestation included egg masses within the city limits of Eugene. In the fall of 1984, the State of California, which constitutes 70 percent of the market for Lane County forest products, imposed a temporary ban on all finished timber and other wood products from Lane County because of the gypsy moth infestation, and Lane County was quarantined by the United States Department of Agriculture. The Agricultural and Resource Economics Department of Oregon State University estimated that greater than a billion dollars of household income and of timber production would be lost annually if the ban continued.[14]

The decision was made to use B.t.-k instead of chemical pesticides for gypsy moth eradication. *Bacillus thuringiensis* var. *kurstaki* was sprayed from helicopters from May 1 through mid-June 1985, over an area with a population of approximately 80,000 persons; in May 1986, it was sprayed over an area that partly overlapped the first area and had a total population of about 40,000 persons. The bacterial product was mixed with a non-ionic surfactant spreader-sticker-carrier matrix prior to application; polyethylene is the active ingredient of this product.

Methods

Laboratory Surveillance

A passive surveillance program, enrolling the four largest clinical laboratories in the area, was developed to evaluate cultures obtained from human specimens during spraying periods. The laboratories included three in hospitals and one in an outpatient setting. All cultures obtained for routine clinical purposes during, and for one month after, the spray period that were positive for any *Bacillus* species were subcultured by the four participating laboratories, and sent to the Oregon State Public Health Laboratory to establish whether any were positive for B.t. *Bacillus thuringiensis* was identified by the presence of toxin crystals (parasporal inclusions) in bacterial suspension smears stained with basic fuschsin.[15] Follow-up information was collected for all B.t.-k-positive specimens, to determine date of collection, fluid or body site cultured, age and sex of patient, the relative amount of *Bacillus* present on primary culture, and the relative amount and identification of other microorganisms present.

A non-sprayed community approximately 60 miles from the spray area was used as a control community during the second spray season. *Bacillus* species found in specimens from that community's principal hospital were also referred to the State Laboratory for identification.

During the second spray season, culture plates were opened to ambient air for 60 minutes at bench tops in each of the laboratories, to assess the degree of air contamination, and the likelihood that a specimen could have become contaminated on that basis. In addition, culture plates were streaked with sterile wire loops, and blood culture bottles were injected with sterile water to simulate laboratory procedures and assess the level of laboratory contamination. One set of these laboratory environmental samples was done before the spray period; one was done during the spray period, and a third set was done on a day when the area near the laboratory or homes of laboratory workers had been sprayed.

In addition to laboratory surveillance, telephone complaints received by the Lane County Health Department from members of the public were tabulated and evaluated to determine whether there were observable patterns of clinical disease complaints in the general population.

Case Definitions

Physicians were asked the date of onset of the condition for which the positive *Bacillus* culture was obtained, patient history or physical findings which prompted the culture, therapy, outcome (particularly as its time course related to antibiotic therapy), final diagnoses, and other questions pertinent to the particular patient and clinical setting, such as underlying disease states.

Criteria for deciding that B.t.-k could be rejected as a pathogen were set in advance; one or more of the following conditions had to be met:

- There was no clinical evidence of infection.
- The onset of the condition for which the culture was obtained predated the spray period.
- There was evidence of another pathogen or etiology that adequately explained the disease.
- The condition responded to an antibiotic to which B.t. is resistant.
- There was an apparently negligible quantity of B.t. present. (Quantity present was not used as a criterion if the specimen was taken from a normally sterile site, such as blood or urine, except for a urine specimen from a patient with an indwelling bladder catheter.)

Results

During the 1985 study period, 60 subcultures of *Bacillus* species from patient cultures were received by the State Laboratory; 42 were identified as B.t. In 1986, 35 additional clinical *Bacillus* isolates were received from Lane County, of which 13 were B.t. In 1986, seven specimens were received from the control laboratory in the non-sprayed community; none was identified as B.t. Specimens positive for B.t. were taken from 18 different body sites or fluids, including five sites expected to be sterile, 10 environmentally exposed sites, and three other sites.

Data were combined for the two study periods, since there were no known systematic differences between the two spray periods, other than the size of the population sprayed.

Of the laboratory environmental samples, no *Bacillus* species were found in any of the sterile loop-streaked plates or the simulated blood culture sets from any of the laboratories. Of 24 60-minute plates exposed in the sprayed community during the spray period, B.t. was found on two. One was from the bench top in the work area of an employee whose home had been recently sprayed. Non-B.t. *Bacillus* isolates grew on five environmental plates. No *Bacillus* was

grown from environmental plate samples from the control community.

Telephone calls received by the Lane County Health Department did not reveal any pattern of predominance of any one symptom complex or of involvement of any single organ system. Symptoms were those common to any community, e.g., nausea, headache/dysphoria, rash, angioedema.

Fifty-two (95 percent) of the 55 B.t. specimens received were assessed to be probable contaminants, either of skin or tissue or of the laboratory plates (Table 1). One of these was from a spray project worker who sustained a splash of B.t. mixture to his face and eyes and developed dermatitis, pruritis, burning, swelling and erythema, with conjunctival injection. B.t. was cultured from his conjunctiva. He was treated with steroid cream to eyelid and skin, with total resolution. For the remaining three patients, B.t. could not be ruled out as a pathogen; their case histories follow.

PATIENT 1: B.t. cultured from blood—This patient was a 77-year-old male who initially presented June 7, 1985 with a diagnosis of superior vena caval syndrome. Computerized tomography of the thorax revealed a right mediastinal mass and a right pleural effusion. The patient underwent bronchoscopy and mediastinoscopy. Bronchial brushings, needle aspirations and mediastinal biopsy were negative for malignant cells. The patient was treated on an ambulatory basis with prednisone, 40 mg. p.o. per day, and furosemide to effect a diuresis; there was some improvement as a result of these measures.

Two weeks later, a thin needle aspirate of the mediastinal mass was found positive for malignant cells. The procedure was complicated by a right pneumothorax, and the patient was hospitalized. A chest tube was inserted twice during this hospitalization. The steroid dose was tapered down to 10 mg per day at the time of discharge, June 30, 1985. He remained afebrile throughout this hospitalization, and his white blood count was within normal limits.

On July 14, 1985 the patient was readmitted with a fever which had begun two days previously, and cough productive of mucoid sputum without purulence or hemoptysis. On admission, he was dyspneic with rales in the left lung. The temperature peaked at 38.8°C four hours after admission. The chest X-ray showed a left upper lobe infiltrate and pleural fluid. Blood cultures were obtained, and the patient was started on gentamicin and cephazolin. On the third hospital day, the laboratory reported a gram positive *bacillus* in the blood culture. The blood cultures had been taken from two separate sites, each of which was cultured both aerobically and anaerobically. One of the four, an anaerobic bottle, was turbid at 24 hours, and grew out B.t. on subculture. The other three remained negative on repeated subculture over the following seven days. No sputum or other pulmonary specimen cultures were obtained.

Cephazolin was discontinued and cefaperazone was begun when the B.t. was found. On the fifth hospital day, vancomycin was added, and cefaperazone was discontinued. The patient's temperature reached or exceeded 38°C on 8 of 13 days in the hospital, including a second peak of 38.5°C three days after vancomycin was started. He developed progressive renal failure and eventually expired 13 days after admission. The family refused autopsy. The attending physician felt that the patient had pulmonary sepsis, and that this was the proximate cause of death.

PATIENT 18: B.t. cultured from gallbladder contents—This patient was a 31-year-old mentally retarded female with a spastic hemiplegia and seizure disorder secondary to bilateral subdural hemorrhages suffered in a motor vehicle accident 10 years prior to this admission, her 14th. She had chronic pyuria, treated with trimethoprim-sulfamethoxizole. She first presented April 13, 1985 with a history of intermittent upper abdominal pain over the past several months. Findings were non-specific, but she continued to have intermittent or chronic abdominal pain, and presented May 18, 1985 with continued exacerbation of the pain. She was afebrile. An abdominal ultrasound demonstrated "multiple gallstones associated with either a common hepatic duct or common bile duct stone as well." White blood cell count on admission was 8900, but increased to 14,500 on the first post-operative day. Operative findings were "acute hydrops of the gallbladder with a stone impacted at the base of the cystic duct and a marked inflammatory response surrounding the same." The pathologic diagnosis was "acute gangrenous cholecystitis with cholelithiasis." The patient recovered uneventfully from surgery.

A gallbladder fluid specimen was cultured in broth and on seven plates, five aerobic and two anaerobic. All plates showed no growth, but on the fifth day the broth culture was positive for B.t. No other organisms were recovered. No bacteria were seen on histologic examination of gall bladder tissue.

PATIENT 54: B.t. cultured from an antecubital abscess—This patient was a 25-year-old female seen June 26, 1985 at an emergency room with "an abscess on the right forearm, secondary to injecting with methamphetamine on Friday, June 21." She gave a history of four months of IV drug abuse. On physical examination, the patient was afebrile and there were multiple needle puncture lesions. There was no axillary lymphadenopathy and the lesion was soft. The abscess was incised and a small amount of mixed clot was removed. Assessment was "hematoma vs evolving abscess, right forearm." Twenty colonies of B.t. grew from the wound culture taken at that time.

The patient returned on July 1, 1985, at which time the area around the abscess was found to be indurated. She was started on a seven-day course of erythromycin, with resolution of the abscess.

Discussion

Of 55 cultures from human specimens positive for B.t., in no case could B.t. infection unequivocally be said to be the cause of the disease which prompted the clinician to take the culture. Evaluation on the basis of the described criteria suggested that B.t. infection was not the cause of disease in 52 (95 percent) of the cases. In three cases, B.t. could not be ruled in or ruled out as the causative organism.

The first of these three cases occurred in an elderly, immunocompromised person with underlying lung disease. A pneumonia appeared to have contributed to the cause of death in this patient. Specific identification of the cause of this pneumonia was not possible because cultures of sputum, tracheal aspirates, or lung tissue were not performed. It cannot be retrospectively determined whether this patient's positive blood culture (one of four bottles from one of two sites) represented bacteremia or contamination of the specimen at time of collection. Contamination in the laboratory is unlikely because the culture medium was noted to be turbid at 24 hours, before it had been subcultured or vented to air. The patient's fever and the infiltrate seen on chest x-ray suggest that the patient had an infection; the positive blood

TABLE 1—Role of B.t. and Criteria for Assessment in Patients from Whom B.t. Was Cultured

Patient	Site or Body Fluid Cultured	Criterion Codes	Assessed Role of B.t. in Patient: Contaminant (probable or known)	Assessed Role of B.t. in Patient: Pathogenicity Not Ruled Out
Normally Sterile Sites or Fluids				
1	Blood	—		x
2	Blood	iv	x	
3	Blood	iii	x	
4	Blood		x	
5	Blood	iii	x	
6	Blood	i	x	
7	Blood	iii	x	
8	Blood	iv	x	
9	Blood	iii	x	
10	CSF	i	x	
11	CSF	iii	x	
12	Urine	i	x	
13	Urine	iii/iv/v	x	
14	Urine	ii/iii	x	
15	Urine	iii/iv	x	
16	Bursa, synovial fluid	i/iii	x	
17	Bursa, synovial fluid	iii	x	
18	Gallbladder	—		x
Environmentally-Exposed Sites or Body Fluids				
19	Skin rash	ii/v	x	
20	Skin rash	ii/v	x	
21	Skin rash	ii/iii	x	
22	Skin rash	iii	x	
23	Skin rash	iii/iv/v	x	
24	Skin lesion	ii/iii/v	x	
25	Skin lesion	iii/iv/v	x	
26	Skin lesion	iii/iv/v	x	
27	Skin lesion	iv	x	
28	Sputum	ii/iii/v	x	
29	Sputum	i/ii	x	
30	Sputum	iv/v	x	
31	Sputum	iii/v	x	
32	Sputum	iii	x	
33	Cervix	i	x	
34	Cervix	iii/iv/v	x	
35	Cervix	ii	x	
36	Throat	ii	x	
37	Throat	iii/v	x	
38	Throat	iii	x	
39	Wound	iii/iv	x	
40	Wound	iii/iv	x	
41	Wound	iii	x	
42	Wound	iii/iv	x	
43	Wound	iii	x	
44	Wound	i/v	x	
45	Wound	iii	x	
46	Ear	ii/iii/v	x	
47	Ear	iii/iv/v	x	
48	Stool	iii	x	
49	Stool	i/v	x	
50	Nasal mucosa	ii/iii/v	x	
51	Conjunctiva	iii	x	
52	Conjunctiva	iii	x	
Other Sites				
53	Amputation site bone marrow	v	x	
54	Abscess	—		x
55	Cellulitis, hematoma	iii/iv	x	

Criterion Codes
i. No suggestion of infection.
ii. Condition predates spray period.
iii. Evidence of another pathogen or etiology which adequately explains the disease.
iv. Response to antibiotic to which B.t. is resistant.
v. Apparently negligible quantity present.

culture suggests that it might have been caused by B.t. However, the observations that three of four blood cultures were negative and that the patient failed to respond to antibiotics to which the B.t. was susceptible suggest that the pulmonary infection may have been caused by a different organism.

In the second case, B.t. was grown from one of eight cultures of gall bladder fluid collected at time of cholecystectomy for acute gangrenous cholecystitis with cholelithiasis. The only evidence that B.t. might have been causing infection in this case is the positive culture and the absence of other organisms on culture. Evidence against infection includes the observation that only one of eight cultures was positive, and that it was not positive until five days after collection. The patient's lack of fever and the negative histologic examination of the gall bladder for bacteria also argue against infection.

In the third case, B.t. was grown from a possible abscess at an injection site in an IV drug user. The B.t. could have been responsible for this localized infection, but it could also have been a skin or wound contaminant, or it could have colonized an abscess caused by another organism.

In evaluating whether B.t. played a pathogenic role in the other 52 cases, it should be noted that specimens received were from 18 different body sites or fluids. This suggests that this *Bacillus* is usually appearing as a contaminant or commensal, rather than a pathogen, since there is no consistent pattern of disease associated with its presence.

More than three times as many cultures were positive for B.t. in the first year of spraying than in the second year, although the population living in sprayed areas in the first year was only twice as large as in the second year. In the first year, one participating laboratory was at the spray boundary, two were within 0.2 miles of the boundary, and one was 1.4 miles away. In the second year, the closest laboratory was one mile from the spray boundary, while the other three were 1.4 miles, 2.0 miles, and 3 miles away, respectively. This suggests an increased potential for contamination of cultures in the laboratory during the first year compared to the second. The minimal environmental sampling performed, however, does not permit evaluation of this possibility.

The criteria used to assess whether B.t. played a pathogenic role in these other 52 patients were deliberately conservative. Nevertheless, it is possible that B.t.-caused disease may have been missed. B.t. as an opportunist may have exacerbated existing disease, and could have been acting as a co-pathogen or synergist in cases where there was another pathogen or etiology which adequately explained the disease.

The criterion that the condition responded to an antibiotic to which B.t. is resistant raises some theoretical questions. One is whether B.t. could cause self-limiting infections such that the antibiotic administered is irrelevant. A second is that the toxin crystal could cause disease without infection. However, there is experimental evidence to indicate that the toxin crystal of B.t.-k does not produce disease in mammals.[16] Protoxin activation of the toxin crystal requires a pH higher than physiologic mammalian pH; but such alkaline conditions could obtain in microenvironments, such as urine in the presence of urea-splitting *Proteus* spp., a gastrointestinal tract therapeutically treated with antacids, or in the disease state achlorhydria. Portions of the normal gastrointestinal tract, such as the contents of the duodenum and the gallbladder, are also alkaline. Finally, it is known that *Bacillus thuringiensis* var. *israelensis* readily loses its plas-

mid that codes for the toxin crystal, and that resistance to penicillin and cephalosporin antibiotics is lost with the loss of the plasmid; therefore, it is possible that B.t.-k could be rendered sensitive to antibiotics by plasmid loss.[12,19,20]

Many of the complaints from the public received by the Lane County Health Department were related to skin rashes, angioedema, eye irritation, and respiratory involvement. It could be argued that these symptoms are more consistent with disease caused by the insect itself than B.t.-k. There have been reports of major outbreaks of dermatitis in the Northeastern United States involving thousands of persons exposed to gypsy moth larvae. Dermatitis in these outbreaks was attributed to irritation by the setae or hairlike projections of the gypsy moth caterpillar. Associated symptoms of workers exposed to the larvae included rhinitis, irritation of the eyes, and dyspnea. Wheal and flare reactions have occurred in response to scratch tests in persons with a history of exposure to gypsy moths.[20,21] Eighty ng/organism of histamine have been extracted from these setae.[17,18]

Bacillus thuringiensis var. *kurstaki* has a remarkable safety record in view of its wide use by gardeners, in agriculture, and for major pest eradication projects such as the one undertaken in Lane County. However, *Bacillus* species found in laboratory cultures are usually considered to be contaminants and are not identified, other than to rule out pathogens or opportunists, such as *Bacillus anthracis*, *B. cereus*, or *B. subtilis*. Identification of the toxin crystal involves a specialized technique, not usually indicated for purposes of medical care.[15] Therefore, B.t. disease could have been missed in persons who had contact with the pesticide in other sprayed areas, simply because the *Bacillus* was not known to be B.t. and was interpreted as a contaminant.

In the 30 years during which B.t. has been in widespread use, there has been an increasing proportion of persons in any community who are immunocompromised on some basis. At the same time, the medical community has become more reluctant to label any bacterium as absolutely non-pathogenic to humans. Perhaps the best example of a pathogen once thought to be non-pathogenic to humans is *Serratia marcescens*, now recognized as one of the 15 most frequently isolated pathogens in association with nosocomial infectious diseases in hospitals.[22]

Bacillus species in general can no longer be dismissed as harmless commensals or skin or air contaminants. The current concept is that any isolate should be considered in its clinical context to decide whether it is a pathogen.[23]

This study raises a fundamental issue to be considered by agencies responsible for regulating the use of microorganisms for pest control. These microorganisms may have potential for causing disease in immunocompromised persons. Therefore, such individuals should be advised on how to use biopesticides and how to protect themselves from undue exposure in areas where they are used. Introductions of other biological agents being considered for pest control should occur only after their safety for the seriously compromised host is evaluated. This is important in light of the increasing potential usefulness of microorganisms for pest control made possible by the new technologies of genetic engineering.[24–26]

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