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Bacillus anthracis contamination and inhalational anthrax in a mail processing and distribution center

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ABSTRACT

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Aims: Four inhalational anthrax cases occurred in a large mail processing and distribution center in Washington, DC, after envelopes containing *Bacillus anthracis* spores were processed. This report describes the results of sampling for *B. anthracis* spores during investigations conducted in October and December 2001.

Methods and Results: Wet swabs, wet wipes, vacuum sock, and air-filter samples were collected throughout the facility to characterize the extent of building contamination. The results showed widespread contamination of *B. anthracis* spores, particularly associated with one delivery bar code sorter (DBCS) machine that had sorted the spore-containing envelopes and an area where the envelopes were handled by postal workers. Spore concentrations decreased as distance from the DBCS machine increased, but spores were widely dispersed into surrounding areas.

Conclusion: The spatial distribution of culture positive samples was closely related to the work areas of the inhalational anthrax cases and supported epidemiological evidence that the workers became ill from exposure to *B. anthracis* spores in areas where the contaminated envelopes had travelled.

Significance and Impact of the Study: The results of this investigation were used to guide decontamination efforts and provided baseline spore concentrations for follow-up measurements after the building had been cleaned. Implementing methods to reduce aerosolization and dispersion of dust within the facility would reduce postal workers' potential exposures to bioterrorism agents.

Keywords: anthrax, *Bacillus anthracis*, bacterial spores, bioterrorism, postal facility, surface sampling.

INTRODUCTION

Before 2001, only 18 cases of inhalational anthrax had been reported in the US in the previous century, with the last reported case in 1976 (Inglesby *et al.* 2002). These cases generally acquired the disease through their work, with 13 of them associated with exposures in or near goat hair mills, wool mills and tanneries (Brachman 1980). Occasional epidemics related to processing highly contaminated imported animal fibres, particularly goat hair, have occurred in industrial settings with cutaneous anthrax being more common than inhalational anthrax.

The largest epidemic reported in the US occurred in 1957 in a goat hair-processing plant (Brachman *et al.* 1960; Plotkin *et al.* 1960). Five cases of inhalational anthrax and

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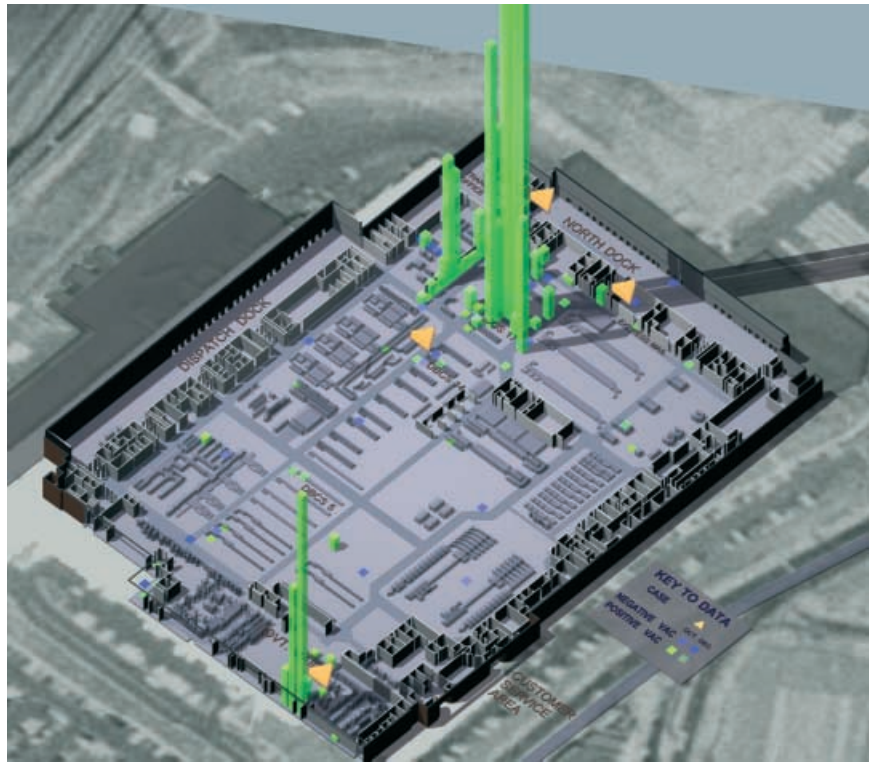

Fig. 1 Primary work locations of inhalational anthrax cases and locations and concentrations of HEPA vacuum samples

four cases of cutaneous anthrax occurred among approx. 600 employees over a 10-week period. None of the cutaneous cases died, but four of the five inhalational cases died within 25 h of the onset of severe symptoms. Investigation of this epidemic showed that over 50% of the samples from goat hair bales imported from Pakistan were culture positive for *Bacillus anthracis* and that each patient had direct contact with this particular batch of goat hair (Brachman *et al.* 1960).

In the anthrax attacks of 2001, *B. anthracis* spores were mailed in at least five envelopes from Trenton, NJ, to locations in New York City and Washington, DC. Eleven inhalational anthrax cases resulted; seven of these cases were US Postal Service (USPS) workers (Inglesby *et al.* 2002; Jernigan *et al.* 2002). These were the first reported cases of inhalational anthrax associated with occupational exposure in the US since the epidemic reported in 1957.

Four cases of inhalational anthrax were diagnosed among employees of a large mail processing and distribution center in Washington, DC, during October 2001; two of the employees died (Dewan *et al.* 2002). This facility was contaminated with *B. anthracis* spores by at least two envelopes mailed to US Senators whose offices were located in the Hart Senate Office Building. The path of these envelopes through this 46 450 sq m facility was reconstructed (Dewan *et al.* 2002). The envelopes entered the building in a standard mail tray on the evening of October 11 or early morning October 12 through a loading dock door near the office where one of the inhalational anthrax cases primarily worked (Fig. 1). The envelopes were first routed through a large tray-sorting machine and then processed through delivery bar code sorter (DBCS) machine 17 between 7:05 and 7:30 AM on October 12. DBCS machines move mail along internal conveyor belts and rollers through a series of turns and compressions at 51 km h^{-1}, sorting the mail into collection bins for distribution. After twice passing through DBCS machine 17, at least one of the envelopes was taken to the government mail section where all envelopes mailed to US government addresses in the DC area are handled manually for distribution. In this area, workers flip through envelopes – a procedure called riffling – to confirm that they were properly sorted by the DBCS machines. One postal worker in the government mail section developed inhalational anthrax (Fig. 1). Finally, this contaminated envelope was placed in a tray, put on an all-purpose-carrier, and loaded on a truck for delivery to the Hart Senate Office Building. The path of the other envelope after it left DBCS machine 17 remains uncertain (Dewan *et al.* 2002).

Twenty DBCS machines were operated at the facility over three shifts every day of the week. Each morning between 7:30 and 10:00 AM they were routinely opened and cleaned by blowing with compressed air to remove dust build-up. The operators of DBCS machine 17 did not develop inhalational anthrax. However, an operator of DBCS

machine 14, which was 27–30 m from DBCS machine 17, and a worker in the express mail room – which had two doors approx. 45 and 55 m from DBCS machine 17 – did develop inhalational anthrax (Fig. 1). These workers may have periodically passed near DBCS machine 17 while going to the restroom, breakroom, or in the course of their work duties.

This report describes the *B. anthracis* spore contamination resulting from processing of the contaminated envelopes within the facility. The environmental evaluation by the Centers for Disease Control and Prevention (CDC) focused on the supposed path of the envelopes through the facility and the locations where the cases worked. However, samples were also collected throughout the facility, including the administrative areas on the second level and the customer service area in the front of the building.

On 18 October – before CDC investigators arrived – a USPS contractor collected dry swab samples for detection of *B. anthracis* spores. Twenty-nine samples were collected around government mail sorting bins and DBCS machines 16, 17, 18 and 19. Fourteen samples tested positive for *B. anthracis* spores – 10 of 17 in the government mail area and four of 12 on the DBCS machines. Both samples collected from DBCS 17 were positive. At the time of sampling, no cases of inhalational anthrax were known among the work force and sampling was concentrated in areas through which one envelope was believed to have passed. The building was evacuated on 21 October after the first worker was confirmed to have inhalational anthrax. The results of the contractor's dry swab samples became available on 22 October and on 23 October CDC investigators – accompanied by USPS contractors – entered the building to begin the investigation.

MATERIALS AND METHODS

During the first CDC survey (23–28 October 2001), samples were collected for *B. anthracis* spores using three techniques – surface wipe, surface high-efficiency particulate air (HEPA) vacuum sock and air-filter sampling. During the second CDC survey (17–20 December 2001) surface swab samples were also used to compare the relative surface sample collection effectiveness of swab, wet wipe and HEPA vacuum sock samples. The results of this comparison were reported separately (Sanderson *et al.* 2002a). Samples were also collected during the second investigation to more fully characterize the *B. anthracis* spore contamination inside the building. The following are descriptions of how each type of sample was collected and analysed during these investigations. Each sample was collected by first donning nonpowdered gloves over the two pairs of nitrile protective gloves that were part of the personal protective equipment worn by each investigator.

Wet swab samples

A sterile rayon swab (Environmental Swab Kit, CDC, Atlanta, GA, USA) was removed from a sterile tube, moistened by inserting into a second tube containing a sponge soaked with 1·5 ml of phosphate-buffered saline (PBS) at pH 7·2, and then used to swab a selected surface by moving the swab back and forth horizontally and then vertically. The swab was rotated during sampling to ensure that the entire surface of the swab was used. After sampling, the swab was returned to its original, prelabelled sampling tube for submission to the laboratory. The area of the surface sampled was measured and recorded in square inches.

Wet wipe samples

Selected surfaces were wiped with a 7·6 × 7·6 cm sterile rayon gauze pad (Dukal Corporation, Syosset, NY, USA) premoistened with about 5 ml of Baxter Sterile Water for Irrigation, USP® (Baxter Healthcare Corporation, Deerfield, IL, USA). The surface was thoroughly wiped back and forth by making three to four vertical strokes, folding the exposed side of the pad, and making three to four horizontal strokes over the same area with the other side of the wipe. The pad was then placed into a prelabelled, 50 ml sterile conical tube and sealed with a cap. During the first survey, the surface area within which samples were collected was not recorded, but during the second survey, the surface area wiped was measured and recorded in square inches.

HEPA vacuum sock samples

A cone-shaped filtering 'sock' (dust collection trap manufactured by Midwest Filtration Co., Fairfield, OH, USA) was inserted into the nozzle of a HEPA vacuum cleaner (Vacomegah manufactured by Atrix International Inc., Burnsville, MN, USA). The vacuum employed a 120 V, 6·6 A, 745 W electric motor to pull 790 l of air per minute through the vacuum nozzle. The plastic sleeve of the dust collection trap was folded over the outside of the nozzle and hand-held in place while the vacuum nozzle was moved slowly back and forth across the sampled surface. The dust collection trap was removed from the vacuum nozzle and placed into a prelabelled, 50 ml sterile conical tube and sealed with a cap. During the first survey, the surface area within which the sample was collected was not recorded, but during the second survey the surface area vacuumed was measured.

Before inserting a clean sock into the vacuum nozzle and collecting a sample, the sample collector put on a new pair of gloves and thoroughly wiped the inside of the vacuum nozzle with an alcohol wipe. An alcohol wipe physically removed contamination from the nozzle surface, but was not

expected to sterilize the surface as alcohol has not been shown to effectively kill *B. anthracis* spores (Alcamo 2000).

Air samples

Air samples were collected open-faced on 37 mm mixed cellulose ester (MCE) filters (0·8 μm pore size) in three-piece polystyrene cassettes attached to sampling pumps operating at 2 l min^{-1}. The samples were placed throughout the facility in fixed locations; sampling durations were approx. 30 h. After sampling, filters were removed from the sampling train, the caps were reattached, and the filters were placed inside clean plastic bags.

Culture analysis

The CDC, National Center for Infectious Diseases (NCID) laboratories in Atlanta, GA, conducted culture analysis of the swab and wipe samples. During the first investigation the wipe samples were extracted by CDC laboratory technicians. Three millilitre of Brain Heart Infusion (BHI) broth were added to the conical tubes containing the wipe samples and the broth and tube were agitated with a wire loop. The tube was heat-shocked for 30 min at 65°C, allowed to cool, then 10–30 μl of the broth were plated on trypticase soya agar (TSA) with 5% sheep blood.

During the second investigation, swab and wipe samples were extracted in a laboratory operated by the USPS contractor at the Brentwood site. In this laboratory, the samples were extracted by adding 30 ml of 0·3% Tween-20 in PBS to a 50-ml Blue Falcon® (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) screw-top tube and vortexing the tube for 3 min. The swabs and wipes were removed and the contents of the tube were allowed to settle for 5 min. The tube was centrifuged at 3731 *g*, for 30 min at 10°C, the supernatant removed by decanting, and the pellet was resuspended in 2 ml of solution. Approximately one half of the resuspended extract was shipped to the CDC Bioterrorism Surge Capacity and Anthrax Laboratories in Atlanta, GA, for culture and confirmatory analysis. The remaining half of the resuspended extract was retained at the site laboratory for analysis by PCR. The results of the PCR analysis were reported separately (Sanderson *et al.* 2002b). At the CDC laboratories in Atlanta, 0·1 ml of the suspension was plated to TSA with 5% sheep blood and streaked for quantification.

In both surveys, the culture plates were incubated at 35–37°C in ambient air and examined after 24 and 48 h. Suspicious colonies were screened using standardized Laboratory Response Network (LRN) level A testing procedures for identification of *B. anthracis* (CDC 2003). Briefly, level A procedures involve isolation of colonies on sheep blood agar. The colonies grow rapidly and are generally flat or slightly convex with edges that are slightly undulate. *B. anthracis* is not β-haemolytic, but is almost always motile. Gram stains show large (1–1·5 × 3–5 μm) Gram-positive rods in chains with oval, central-to-subterminal spores. Identification of all strains was confirmed using standard microbiological procedures and the LRN testing algorithm (Logan and Turnbull 1999; Khan *et al.* 2000). In the first investigation, samples were reported only as culture positive or negative for *B. anthracis* colonies. However in the second investigation, the number of CFU per plate was also reported. To estimate the number of CFU per sampled surface area, the number of CFU per plate were multiplied by 20 (2 ml of extract solution divided by 0·1 ml of plated solution) and divided by the recorded surface area in square centimetres. When the number of colonies on culture plates exceeded about 300, the samples were reported as too numerous to count (TNTC).

In both investigations, the HEPA vacuum sock samples were analysed by a contract laboratory included in the CDC LRN. The HEPA vacuum socks and their contents were weighed on a precision balance. The average weight of five unused sock samples was used to estimate the presample weight of the vacuum socks; the average weight of these unused socks was 0·70 g (S.D.: 0·02 g). The average weight was subtracted from the postsample weight of each sock sample to estimate the weight of its contents. Approx. 20–30 ml of 0·3% Tween-20 in PBS was added to a cup containing the sock and its contents and placed on a shaker for 30 min. The contents of the cup were allowed to settle for 5 min and then the supernatant was poured into a 50-ml Blue Falcon® screw-top tube. The tube was centrifuged at 1658–3731 *g*, for 15–30 min at 10°C, then approx. nine-tenths of the starting volume were removed. The pellet in the bottom of the tube was resuspended in approx. 2 ml of 0·3% Tween-20® in PBS and 0·1 ml (two drops from a Pasteur pipette) and 0·01 ml (using a calibrated loop) of the suspension was plated respectively to two TSA plates with 5% sheep blood and streaked for quantification. The plates were incubated and analysed following the LRN testing and confirmation procedures. The results of these samples were reported as number of CFU per gram of material collected; the estimated weights of the sock contents were also reported. To estimate the number of CFU per sampled surface area, the reported CFU per gram were multiplied by the weight of sock contents and divided by the surface area sampled.

The air samples collected during the first investigation were also analysed by a CDC contract laboratory. The MCE filters were removed from the air sampling cassettes with sterile forceps and placed into a 50-ml Blue Falcon screw-top tube; 20 ml of 0·3% Tween-20 in PBS were added and placed on a shaker for 30 min. The filters were extracted and analysed in the same manner as the HEPA vacuum sock samples. The results of these samples were reported as positive or negative for *B. anthracis* isolates.

Personal protective equipment

When investigators entered the facility to conduct sampling they wore Saranex® (Dow Chemical, Midland, MI, USA) full-body protective suits with hoods, disposable rubber boots, two pairs of nitrile gloves, and full-face powered air-purifying respirators with high-efficiency filters. The gloves and boots were taped to the cuffs of the Saranex® suit, while the respirators were taped to the hood and collar. Investigators exercised care in the use of chemical-resistant gloves to avoid the development of detrimental effects on the skin that can result because of occlusion, sweating and maceration (Wigger-Alberti and Elsner 1998). The respirator facepieces worn by investigators were assigned based on quantitative fit testing. When the investigators exited the building they passed through a decontamination procedure, which included being sprayed twice from head to foot with a 10% solution of sodium hypochlorite followed by a water rinse before removing their suits, boots, gloves and finally respirator.

RESULTS

A total of 114 surface wipe samples were collected during the first survey; eight (7%) of these samples were culture positive for isolates of *B. anthracis* spores (Table 1). A total of 39 HEPA vacuum sock samples were collected during the same survey and *B. anthracis* was isolated in 27 (69%) of these samples (Table 1). The results of HEPA vacuum sock sampling indicated that *B. anthracis* spore contamination was more widespread than the wipe sampling indicated.

During the first survey, 12 air samples for airborne *B. anthracis* spores were collected throughout the facility for approx. 30 h each. All of the air samples were culture negative for *B. anthracis*, indicating that no spores were in the air during the sampling period. The building had been evacuated and the ventilation system not operating for 3 days before the air sampling began.

The culture analysis of the wet swab, wet wipe and HEPA vacuum sock samples collected during the second survey are also presented in Table 1. While 58 of 67 (87%) of the wipe samples and 49 of 59 (83%) of the HEPA vacuum sock samples were culture positive, only 36 of 67 (54%) of the wet swabs were culture positive. The concentration of *B. anthracis* spores in these samples and the HEPA vacuum sock samples from the first survey are reported as CFU per sample, because the surface area over which samples were collected was not recorded during the first survey. These results should only be considered semi-quantitative and the absolute concentrations not directly compared across the sampling methods. The calculated concentrations of *B. anthracis* spores per sample in the culture positive HEPA vacuum sock samples tended to be greater than in the other types of samples; however, culture results for the swab and wipe samples were sometimes reported as TNTC such that their upper count estimate was only about 300 CFU, whereas the concentrations of the HEPA vacuum sock samples were not so constrained.

A greater proportion of the wipe and HEPA vacuum sock samples were positive in the second survey than in the first, but this was expected since a major objective of the second survey was to compare the effectiveness of the three surface sampling techniques (Sanderson *et al.* 2002a). Therefore, sampling was largely conducted in areas thought to be heavily contaminated with *B. anthracis* spores to increase the likelihood of getting positive results. In addition, between the first and second surveys, improvements were made in the technique used to extract spores from wipe samples. During the first investigation, the wipe samples were placed in a conical tube along with 3 ml of BHI broth and simply agitated with a sterile wire-loop. During the second investigation, 20–30 ml of PBS were added to the wipes and they were vortexed for 3 min before centrifuging them to concentrate the spores in the extracting solution.

The results of *B. anthracis* spore sampling from both surveys are presented by location in Table 2. The greatest

Table 1 Results of sampling for *Bacillus anthracis* spores by sample type within survey

	Method	No. of samples tested	*B. anthracis* detected *n* (%)	Median* (CFU/sample)	Range* (CFU/sample)	Level†			
						Neg.	Low	Med.	High
First survey	Wipe	114	8 (7)	ND	ND	106	ND	ND	ND
23–28 October 2001	HEPA vacuum	39	27 (69)	11 400	3–13·3 × 10^6	12	5	6	16
	Air	12	0	–	–	12	0	0	0
Second survey	Wet swab	67	36 (54)	3	1 to >300	31	26	1	9
17–20 December 2001	Wipe	67	58 (87)	200	1 to >300	9	20	10	28
	HEPA vacuum	59	49 (83)	25 000	80–49·6 × 10^6	10	1	4	44

*For positive samples only.

†Level of *B. anthracis* (CFU): negative = 0, low = 0–100, medium = 100–300 and high = >300.

ND = not determined.

Table 2 Results of sampling for *Bacillus anthracis* spores by location within postal facility samples collected during both investigations (23–28 October and 17–20 December 2001)

Location	Wet swab samples			Wet wipe samples			HEPA vacuum sock samples		
	N (% Pos)	Median (CFU)	Maximum (CFU)	*N* (% Pos)	Median (CFU)	Maximum (CFU)	*N* (% Pos)	Median (CFU)	Maximum (CFU)
DBCS machine 17	13 (93)	>300	>300	19 (100)	>300	>300	8 (100)	468 000	$49{\cdot}6 \times 10^6$
Other DBCS machines	10 (20)	3	3	54 (22)	15	200	14 (64)	352	$1{\cdot}39 \times 10^6$
Within 15 m of DBCS machine 17	NS	–	–	9 (11)	–	–	3 (100)	22 400	$4{\cdot}82 \times 10^6$
Secure area – 23 m from DBCS machine 17	18 (72)	4	250	18 (94)	300	>300	18 (100)	22 600	102 000
Loading dock and vehicle transportation office	NS	–	–	11 (0)	–	–	4 (50)	194	348
Express mail room	NS	–	–	3 (0)	–	–	2 (50)	–	200
Government mail area	1 (0)	–	–	17 (6)	–	100	9 (100)	17 900	13×10^6
Other locations in mail processing area	3 (0)	–	–	20 (0)	–	–	15 (67)	550	19 600
Administration and customer service area	NS	–	–	10 (0)	–	–	5 (0)	–	–

N, number of samples collected; CFU, colony forming units per sample; NS, not sampled.

proportion of positive samples and highest concentrations of *B. anthracis* spores were found in work areas associated with the path of at least one of the contaminated envelopes through the facility – along DBCS machine 17 and in the government mail area. Although the concentrations tended to decrease with distance from DBCS machine 17 and the government mail area, spores were also found in areas relatively far from these work areas.

Ninety-three per cent of the swab samples and 100% of the wipe and HEPA vacuum sock samples collected on DBCS machine 17 were culture positive for *B. anthracis* spores. These samples were collected inside machine 17, on its control console and letter feeder, on the dust collector above the letter feeder, and in numerous sorting bins. Among the highest spore concentrations were those from samples collected inside this machine and its sorting bins. These results show that although the machine had been cleaned by HEPA vacuums and washed with sodium hypochlorite solution after the first survey, *B. anthracis* spores were still present; often, the number of CFU in the wet swab and wipe samples were reported as TNTC. Bin 4 was the bin to which one of the envelopes was suspected to have been sorted, while bin 139 was the bin to which the other envelope was suspected to have been sorted initially. Although the concentrations of spores detected via wet swab and wipe samples for both of these bins were reported as TNTC, so were the results for several other bins; these measurements could not confirm that these envelopes had landed in any particular sorting bin. Therefore, it appears that the entire machine was heavily contaminated by dispersion of the spores through the sorting process, compressed air blowing of the machine, or the cleaning process. In addition, samples collected on a ventilation diffuser and inspector window portals approx. 5 m directly above DBCS machine 17 were culture positive.

Approximately 20% of the swab and wipe samples and over 60% of the vacuum samples collected from other DBCS machines were culture positive for *B. anthracis* spores. Although all concentrations were <3 CFU cm^{-2}, wipe and vacuum samples collected inside and on DBCS machine 14, where one of the inhalational anthrax cases had worked, were culture positive. Swab, wipe and vacuum samples collected inside and on machines 5, 16, 18, 19 and 20 were culture positive and most of the spore concentrations in these samples were <3 CFU cm^{-2}. Because envelopes containing *B. anthracis* spores were not known to have passed through these machines, they may have been cross-contaminated by aerosolization of spores from machine 17 or contact with other envelopes or materials, which had contacted the spore-containing envelopes.

Samples collected on surfaces near DBCS machine 17 – a time clock, compressed-air hose housing, and other mail processing machines – were culture positive for *B. anthracis* spores. The concentrations of spores were lower than those found on machine 17, but clearly indicated that large numbers of spores had been aerosolized into the work areas surrounding this machine. The spore concentrations were high on 18 sets of samples – swab, wipe and HEPA vacuum sock samples – collected side by side on the tops of sorting bins along the wire cage of a secure mail area 23 m from DBCS machine 17. None of the HEPA vacuum sock samples and only one of the wipe samples was negative. This area had not been vacuumed and washed with sodium hypochlorite solution and there was a substantial amount of dust on top of these bins. As workers did not routinely touch the tops of these bins during mail processing, they were most likely contaminated via aerosolization and dispersion of the spores.

One of the inhalational anthrax cases worked in the vehicle transportation office and along the loading dock, and

drove a USPS vehicle. No wipe samples collected in any of these areas were culture positive for *B. anthracis* (Table 2). However, HEPA vacuum sock samples collected from the carpet in the vehicle transportation office and from an electrical box on the loading dock were positive, but the levels (maximum 120 CFU g^{-1}) were considerably lower than those found on or near DBCS machine 17. Three wipe samples collected inside the Express Mail room where another inhalational anthrax case worked were negative, but one of two HEPA vacuum sock samples was culture positive (28 CFU g^{-1}). Again, the spore concentration on this sample was much lower than those found on or near DBCS machine 17. These samples indicated that the areas where these individuals worked were contaminated by *B. anthracis* spores, although probably at levels much lower than encountered near DBCS machine 17.

Sixteen wipe samples collected in the government mail area during the first survey were all negative, despite the fact that 10 of 14 swab samples collected 1 week before by a USPS contractor were confirmed positive by CDC and had been collected in virtually the same locations. This supported the concern that the technique used to extract spores from the wipes during the first survey was deficient. All HEPA vacuum sock samples collected in the government mail area were positive, indicating widespread, relatively heavy contamination in this area (Table 2). The greatest concentration of spores found within the building was in the government mail area – 13 300 000 CFU on a HEPA vacuum sock sample collected on top of the sorting bin where the inhalational anthrax case from the government mail area had worked. Reportedly, this worker rarely moved outside this area.

The HEPA vacuum sock samples collected in other mail processing locations farther from DBCS machine 17 and the government mail area were culture positive for *B. anthracis* spores (Fig. 1). These areas may have become contaminated via aerosolization of spores from other areas or direct contact with contaminated mail.

Five wipe samples and three HEPA vacuum sock samples were collected in the administration area on the second level and five wipe samples and two HEPA vacuum sock samples were collected in the customer service area. All samples taken in the administration and customer service area were culture negative (Table 2).

During the second investigation, side-by-side sample sets – swab, wipe and HEPA vacuum sock – were collected from window portals along the postal inspector walkways directly above DBCS machine 17 and then from walkways at varying distance from the machine. These portals were approx. 5 m above floor level and were covered with long-term settled dust. These portals were not easily accessed and could only have been contaminated by aerosolization and dispersion of spores. Samples from portals directly above DBCS machine 17 were heavily contaminated with *B. anthracis* spores (Table 3). As the distance from the machine increased, the concentration of spores decreased. Once the distance exceeded 60 m, the concentration of spores dramatically decreased. No spores were detected on portals, which were more than 90 m from DBCS machine 17.

The results of the HEPA vacuum sock samples (in CFU g^{-1}) and the primary work locations of four employees who developed inhalational anthrax are presented in Fig. 1. The spore concentrations by sample site are shown by the height of the bars. This figure shows that the greatest concentration of *B. anthracis* spores were found around DBCS machine 17 and the government mail area where the contaminated envelopes were processed. Work areas near DBCS machine 17 were also heavily contaminated, probably by aerosolization of spores during mail processing and blowing with compressed air. Culture positive samples were

Table 3 Results of sampling for *Bacillus anthracis* spores on inspector walkway portals samples collected during second investigation (17–20 December 2001)

Location		Samples (*n*)	Positive (*n*)	TNTC (*n*)	Median (CFU in^{-2})	Range (CFU in^{-2})
Directly above DBCS machine 17	Wet swab	3	3	0	58	29–233
	Wipe	3	3	3	18	18
	HEPA vacuum	3	3	NA	310	239–322
Within 30 m of DBCS machine 17	Wet swab	5	2	0	0	0–9
	Wipe	5	5	2	16	1–233
	HEPA vacuum	5	5	NA	67	6–264
Within 30–60 m of DBCS machine 17	Wet swab	8	6	0	0	0–58
	Wipe	8	7	1	7	0–18
	HEPA vacuum	8	7	NA	10	0–60
>60 m from DBCS machine 17	Wet swab	4	0	0	0	NA
	Wipe	4	1	0	0	0–15
	HEPA vacuum	4	1	NA	0	0–2

TNTC, too numerous to count; NA, not applicable.

found in other areas of the building, but the concentrations on these HEPA vacuum sock samples were much lower.

DISCUSSION

The results of these two surveys indicated widespread contamination of *B. anthracis* spores within a large mail processing and distribution center in Washington, DC. Contamination was particularly associated with one DBCS machine, which had mechanically sorted spore-containing envelopes and an area where envelopes were handled before distribution. Spore concentrations decreased as distance from the DBCS machine increased, but spores were still widely dispersed into areas surrounding this machine – particularly towards the loading dock and the north-west corner of the building. Three of the four employees who developed inhalational anthrax worked in this area. The other employee who developed inhalational anthrax worked in the government mail area, which was also heavily contaminated. No sample collected in the second level administrative area or the customer service area was positive for *B. anthracis* spores.

Of the 2403 individuals employed in the facility, 1961 of them worked in the nonpublic area of the building processing mail and 442 of them worked in the administration and customer service areas where no *B. anthracis* spores were found. The four workers who developed inhalational anthrax all began to have symptoms 4 days after the letters were processed, and all worked in the nonpublic, mail processing area of the building (attack rate 2·04 per 1000 persons). One inhalational anthrax case worked in government mail, where 350 workers were employed over all workshifts. The primary work stations of three inhalational anthrax cases were within 60 m of DBCS machine 17, and they may have frequently passed through areas heavily contaminated by aerosolized spores. As spore contamination was widespread and spores were probably re-aerosolized repeatedly by mail processing and other work activities, workers on every shift were at risk of exposure, not just employees working when the contaminated letters were processed.

All of the 1961 workers in the nonpublic, mail processing area of the building could have been exposed to *B. anthracis* spores, but only four developed disease. Many of the other workers may not have developed disease because they had not received a sufficient dose of spores. However, at least some of these workers were potentially exposed to many thousands of spores and may have eventually developed the disease if they had not begun taking antibiotics 10 days after first exposed.

Spores remained on machine 17 and machines nearby, although their surfaces had been cleaned by HEPA vacuums and washed with sodium hypochlorite solution, and over 2 months had passed since the envelopes containing *B. anthracis* spores passed through the facility. Clearly, *B. anthracis* spores had survived vacuuming and washing. This initial cleaning was not expected to totally remove the spores from contaminated areas, but simply reduce the number of spores. Fumigation methods were under testing at the facility to destroy the viability of remaining spores.

Three inhalational anthrax cases worked in the general area near DBCS machine 17 and were exposed to *B. anthracis* spores aerosolized during mail processing or by the use of compressed air to clean the machine. The other DBCS machines may have been contaminated by envelopes passing through them, which had contacted the envelopes, which were known to contain *B. anthracis* spores – secondary or cross-contamination. It is also possible that other envelopes containing *B. anthracis* spores, which remain unknown, were processed by these machines.

It was not clear why so few wipe samples were culture positive during the first investigation, compared with the second one. During the first survey, it is possible that wipe samples were more frequently collected on uncontaminated or lightly contaminated surfaces, but during the second survey more heavily contaminated surfaces were sampled. In areas, which were more heavily contaminated over a broad surface – such as DBCS machine 17 – an adequate number of spores may have been present for detection, but in areas, which were lightly contaminated, fewer spores were available for detection. However, it is also possible that the spores were not adequately extracted from the wipe samples during the first survey.

Surface sampling has inherent limitations. If sample collectors avoid cross-contamination of the samples and laboratory personnel accurately identify *B. anthracis* colonies, false positive samples should not occur. However, the number of false negative samples may be great if *B. anthracis* spores are not adequately collected from surfaces, extracted from the sample media, and properly cultured and analysed. In addition, because it is not practical to sample all surfaces within a building, some surfaces containing anthrax spores might be missed. The errors present in sampling and analysis may – at least partially – be the cause of differences seen between the two surveys.

Where spore contamination is heavy, surface samples may be able to collect a sufficient number of spores to be extracted, cultured and positively identified. But where contamination is comparatively lighter, the samples may miss them either because they are not effective at picking them up or cover a comparatively small surface area. Vacuum samples are able to cover a broader surface area than wipe and swab samples, and collect a greater mass of material than the wipes, resulting in greater sensitivity for the vacuum samples. In addition, as only a portion (approx. 10%) of the total extract was placed on culture plates for analysis, it is possible that extracted spores remained in solution and went unplated, particularly in samples collected

from lightly contaminated surfaces. By plating only a portion of the extract, the true surface concentration may be underestimated for even heavily contaminated surfaces. Therefore, the entire extract should be plated for analysis.

The air samples did not detect any airborne spores during the sampling period, but these samples were collected 12 days after the contaminated envelopes had passed through the building and 3 days after it had been evacuated and the ventilation system turned off. They do not represent conditions inside the post office as mail was processed and contaminated machinery was blown with compressed air. They may indicate that, at least within a few days, the airborne spores had settled out of the air. It is also possible that the air sampling method was not appropriate for collecting airborne *B. anthracis* spores. Or, the air samplers may have collected airborne spores, but the spores may not have been extracted from the filters. Other investigators have shown that the Andersen viable bioaerosol sampler may have superior sensitivity for collecting airborne *B. anthracis* spores (Dull *et al.* 2002; McCleery *et al.* 2002).

The results of this investigation were used to guide decontamination efforts and serve as a baseline for follow-up measurements after the postal building had been cleaned. The results of the second survey in December 2001 showed that more aggressive cleaning procedures needed to be employed. The building was decontaminated and workers returned in December 2003, 26 months after the building became contaminated with *B. anthracis* spores. The spatial distribution of positive samples was closely related to work locations of the cases of inhalational anthrax and supported the epidemiological conclusions that these workers became ill because of exposure in areas where the two spore-containing envelopes were known to have been processed. The sampling and analytical techniques used in this investigation provide reference for evaluations of future bioterrorism attacks.

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