Evaluation of Potential Employee Exposures to *Mycobacterium tuberculosis* at an Elephant Refuge

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The cover photo is a close-up image of sorbent tubes, which are used by the HHE Program to measure airborne exposures. This photo is an artistic representation that may not be related to this Health Hazard Evaluation. Photo by NIOSH.
Highlights of this Evaluation

The Health Hazard Evaluation Program received a technical assistance request from officials at a state health department. They asked us to evaluate the ventilation systems, work practices, and personal protective equipment used by employees of a refuge where elephants with active tuberculosis disease were kept.

What We Did

- We made four site visits between September 2010 and September 2012.
- We interviewed 27 refuge employees. We also reviewed pertinent health records.
- We used smoke and tracer gas to test the ventilation systems in the elephant barns.
- We measured pressure differences in one of the elephant barns.

What We Found

- The employee screening program for tuberculosis disease and the respiratory protection program needed improvement.
- Refuge managers made ventilation and work practice changes on the basis of our recommendations during our evaluation.
- The phase 1 elephant stall area was best ventilated by running the exhaust fans, opening the elephant doors at one end, and closing all other doors.
- In the phase 2 quarantine barn installing new ventilation systems and sealing wall openings stopped the air from moving from the elephant stalls into the surrounding areas. As a result employees no longer needed to wear a respirator in the food preparation area.

What the Employer Can Do

- Treat all elephants with active tuberculosis disease to reduce exposures to employees.
- Turn on the barn’s exhaust fans 15 minutes prior to any employees entering the area.
- Move the door control switches in the phase 1 isolation barn to the outside.
- Check air pressure indicators in the phase 2 quarantine barn daily when elephants with known or suspected TB disease are present and log the results.
What Employees Can Do

- Get a tuberculin skin test at least every year if your last skin test was negative or you have not been tested.
- Get a medical evaluation for tuberculosis disease every year if you had a positive tuberculin skin test in the past.
- Keep the food preparation doors closed except when leaving this area.
- Limit staff in the food preparation area when elephants are in the stall area.
- Immediately report any ventilation system problems to your supervisor.
- Wear a respirator when working within 25 feet of an elephant with confirmed or suspected tuberculosis disease to reduce possible risk to tuberculosis.
- Wear a respirator when entering the phase 1 isolation or phase 2 quarantine barn elephant stall area, even if elephants are not present.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACH</td>
<td>Air changes per hour</td>
</tr>
<tr>
<td>B&amp;K</td>
<td>Brüel and Kjær</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
</tr>
<tr>
<td>HEPA</td>
<td>High efficiency particulate air</td>
</tr>
<tr>
<td>Innova</td>
<td>LumaSense™ Technologies Innova 1312 Photoacoustic Multigas monitor</td>
</tr>
<tr>
<td>MIRAN</td>
<td>Thermo Scientific MIRAN SaphlRe Portable Ambient Analyzers</td>
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<tr>
<td>NIOSH</td>
<td>National Institute for Occupational Safety and Health</td>
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<td>OSHA</td>
<td>Occupational Safety and Health Administration</td>
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<tr>
<td>PAPR</td>
<td>Powered air-purifying respirator</td>
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<tr>
<td>PPE</td>
<td>Personal protective equipment</td>
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<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>TST</td>
<td>Tuberculin skin test</td>
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<tr>
<td>USAHA</td>
<td>United States Animal Health Association</td>
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<td>USDA</td>
<td>United States Department of Agriculture</td>
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</tbody>
</table>
Introduction

The Health Hazard Evaluation Program received a request from a state health department about potential employee exposure to tuberculosis at an elephant refuge. Some of the elephants had known or suspected tuberculosis (TB) disease. We made four site visits between September 2010 and September 2012. During these visits we met with refuge managers and employees; observed workplace conditions, work processes and practices; and evaluated ventilation systems in barns. In May 2011 we observed changes in work practices and ventilation made on the basis of our earlier recommendations. We sent interim letters to the state health department and the refuge managers and employee representative in March 2011, December 2011, April 2012, and October 2013. These letters contained interim engineering, administrative, and personal protective recommendations to the refuge managers. Copies of these letters are available upon request to the National Institute for Occupational Safety and Health (NIOSH).

Site Description

At the time of the evaluation, the refuge occupied about 2,700 acres and had a staff of approximately 30. Most of the employees worked on the refuge grounds around elephants, but some administrative employees worked in a building that was outside the refuge. Several employees lived in houses on the refuge grounds. Dogs and cats lived at the refuge and were in all of the elephant barns. The refuge was generally closed to the public, but tours to certain areas of the refuge were given to people who provided donations.

Four barns housed elephants at the refuge. The adjacent phase 1 isolation barn and phase 2 quarantine barn were on the east side of the refuge. The New Asian and African barns were on the west side of the refuge and were not adjacent. Table 1 provides more information on the size of these barns, the ventilation systems, and the TB disease status of the elephants.
<table>
<thead>
<tr>
<th>Location</th>
<th>Size</th>
<th>Elephants</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1 isolation barn</td>
<td>2,000 ft²</td>
<td>Two of three elephants were diagnosed with active TB disease. One was diagnosed before our evaluation started, while the other was diagnosed during our evaluation. Both were on antimycobacterial treatment during our evaluation.</td>
<td>Two elephant stalls with general rooftop exhaust ventilation fans.</td>
</tr>
<tr>
<td></td>
<td>barn on 6 acres</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 2 quarantine barn</td>
<td>6,000 ft²</td>
<td>Four elephants were initially under quarantine in 2010. One died in 2010. Two elephants were diagnosed with active TB disease over the course of this evaluation. Both elephants were undergoing antimycobacterial treatment during our evaluation.</td>
<td>Barn interior included six elephant stalls with support and maintenance areas, an employee office and break area, and a food preparation area (Figure 1). The elephant stalls had wall-mounted general exhaust ventilation fans. The office and break room had a recirculating heating, ventilating, and air-conditioning system with an ultraviolet germicidal irradiation air cleaning unit.</td>
</tr>
<tr>
<td></td>
<td>barn on 200 acres</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Asian barn</td>
<td>16,000 ft²</td>
<td>One of six elephants had received treatment for active TB disease in 2005. TB disease was not diagnosed in the other elephants during our evaluation.</td>
<td>Barn interior included nine elephant stalls, a food preparation area, and data entry workstations. General exhaust fans were only in the elephant stall area.</td>
</tr>
<tr>
<td></td>
<td>barn on 2,200 acres</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African barn</td>
<td>10,000 ft²</td>
<td>TB disease was not detected in the two elephants during our evaluation.</td>
<td>Barn interior included five elephant stalls, food preparation area, and data entry workstations. There was no mechanical ventilation system.</td>
</tr>
<tr>
<td></td>
<td>barn on 300 acres</td>
<td></td>
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</tbody>
</table>
Tuberculosis

Tuberculosis is a disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) bacteria that can be spread from person to person through the air. TB usually infects the lungs, but it can also infect other body parts such as the brain, kidneys, or spine. Persons with latent TB infection have TB bacteria in their bodies without any obvious clinical symptoms. These persons do not have symptoms of TB disease, and they cannot spread the TB bacteria to others. However, they may develop TB disease in the future but can be treated to prevent this from happening. Persons with TB disease are sick from active TB bacteria when the bacteria are multiplying, which destroys tissue in their body. They usually have symptoms of TB disease and are capable of spreading TB bacteria to others.

From 1997–2011, among captive elephants in the United States, the median point prevalence of *M. tuberculosis* among Asian elephants was estimated to be 5.1%, with a range of 0.3% to 6.7%, while that for African elephants was 0 [Feldman et al. 2013]. It is possible for *M. tuberculosis* to be transmitted from elephants to humans [Murphree et al. 2011], and suspected transmission of *M. tuberculosis* between elephants and humans has been described at other elephant care facilities [Michalak et al. 1998; Davis 2001; Oh et al. 2002]. In this refuge, an investigation by the state health department found evidence of elephant to employee transmission of *M. tuberculosis* in nine employees from 2006–2009. Risk for transmission was increased for elephant caregivers and administrative employees working in the quarantine barn. The administrators who worked in this area had no direct contact with elephants. Indirect exposure to aerosolized *M. tuberculosis* (such as during sweeping, shoveling, or using high pressure water sprays) and delayed or inadequate infection control practices were believed to have contributed to transmission [Murphree et al. 2011]. The administrative staff was moved to an office outside of the refuge before our first visit.
Recommendations to prevent *M. tuberculosis* transmission to employees in the elephant care environment are available [Davis 2001; USAHA 2010]. Guidance from the United States Animal Health Association (USAHA) [USAHA 2010] for testing and treating elephants with TB has been adopted by the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service [USDA 2012]. These guidelines require that all elephants in the United States be tested annually for TB disease. Updated draft guidelines have been published by USAHA [2012] for consideration by USDA Animal and Plant Health Inspection Service.

TB disease in elephants can be diagnosed with a trunk wash procedure and culture [USAHA 2010]. Experts believe this test is the best way to diagnose active TB disease in elephants, but false negative results can occur [Mikota et al. 2001; Lyashchenko et al. 2006; Greenwald et al. 2009; Murphree et al. 2011]. Two blood tests, the Stat-Pak and the MultiAntigen Print ImmunoAssay™, can also be used to diagnose TB disease in elephants. Because the Stat-Pak test is not specific to *M. tuberculosis*, it is often used for initial screening [USAHA 2010]. The MultiAntigen Print ImmunoAssay antibody test is usually used as a follow-up to the Stat-Pak to confirm exposure because it is specific to *M. tuberculosis* [USDA 2012]. Other diagnostic tests for TB disease in humans, including the intradermal tuberculin test and acid-fast smears, are not reliable in elephants [USAHA 2010].

**Methods**

Our objectives in this evaluation were the following:

1. Evaluate employee work practices and use of personal protective equipment (PPE).
2. Evaluate ventilation and air migration patterns within the elephant barns.
3. Evaluate employee work histories and their known TB exposures.
4. Review records on employee TB screening, PPE use, and training.

**Walk-through Surveys and Personal Protective Equipment Review**

We conducted four site evaluations (Table 2). We reviewed written protocols on PPE use and the respiratory protection program for refuge employees.
Table 2. Walk-through evaluations

<table>
<thead>
<tr>
<th>Date(s) of visit</th>
<th>Location(s)</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 2010</td>
<td>Phase 1 isolation barn</td>
<td>Walk-through surveys, measuring barn spaces</td>
</tr>
<tr>
<td>and October 2010</td>
<td>Phase 2 quarantine barn</td>
<td>Ventilation smoke assessment</td>
</tr>
<tr>
<td></td>
<td>New Asian barn</td>
<td>Observe employee work practices and PPE use</td>
</tr>
<tr>
<td></td>
<td>African barn</td>
<td></td>
</tr>
<tr>
<td>May 2011</td>
<td>Phase 1 isolation barn</td>
<td>Walk-through surveys</td>
</tr>
<tr>
<td></td>
<td>Phase 2 quarantine barn</td>
<td>Review progress of engineering changes in barns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Review changes in PPE use and work procedures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>during elephant necropses</td>
</tr>
<tr>
<td>September 2012</td>
<td>Phase 1 isolation barn</td>
<td>Sulfur hexafluoride tracer gas tests</td>
</tr>
<tr>
<td></td>
<td>Phase 2 quarantine barn</td>
<td>Air pressure tests</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Confidential medical interviews of employees</td>
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<tr>
<td></td>
<td></td>
<td>Review of PPE procedures</td>
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</tbody>
</table>

**Review of Clothing and Boot Written Protocols**

We reviewed written protocols on clothing and boot requirements for employees working in the New Asian, African, quarantine and isolation barns, maintenance and contract workers, and refuge directors.

**Ventilation Smoke Assessment**

During the first visit, we used a Rosco Model 1500 machine to generate theatrical smoke in the following locations to observe airflow patterns and evaluate the ventilation systems.

- **Phase 1 isolation barn.** We checked if ceiling-mounted exhaust fans were exhausting air from the elephant stalls. We tested under three conditions: (1) all doors and windows open with the fans on (summer condition), (2) all doors and windows open with the fans off (natural ventilation, summer condition), and (3) all doors closed and two windows slightly opened (winter condition). We also tested one of the exhaust fans with the high efficiency particulate air (HEPA) filter removed in the winter condition set-up to determine if exhaust airflow could be increased. These HEPA filters were placed between the fan grill and fan prior to our first visit to reduce the spread of *M. tuberculosis* in exhaust air from the barn.

- **Phase 2 quarantine barn.** We checked if air was moving between the elephant stall areas and the food preparation area, bathrooms, computer work room, and break rooms (Figure 2). We tested this area under several conditions by varying the use of the exhaust fans and opening and closing doors.

- **New Asian barn.** We checked if air was moving from the elephant stall area into keeper areas. We tested these areas under several conditions by varying the use of the exhaust fans and opening and closing doors.
Tracer Gas Ventilation Assessment

During our final visit to the refuge in September 2012, and after the refuge had implemented our recommendations to improve the ventilation systems, we used sulfur hexafluoride tracer gas to evaluate these systems by characterizing air migration patterns and calculating air exchange rates. Sulfur hexafluoride is an odorless, colorless gas with a NIOSH recommended exposure limit and Occupational Safety and Health Administration (OSHA) permissible exposure limit of 1,000 parts per million [NIOSH 2010]. The target concentrations generated during our evaluation were approximately 25 parts per million. No other sources of sulfur hexafluoride gas were present.

We completed tracer gas tests in the phase 1 isolation barn and phase 2 quarantine barn. In the phase 2 barn, we conducted two tracer gas containment tests of the newly sealed wall that separated the elephant stall area from the support and maintenance areas. We also conducted tracer gas concentration decay analyses in the phase 1 isolation barn and phase 2 quarantine barn. This included measuring the decrease in the tracer gas concentrations in these barns to see how quickly the ventilation system was able to purge the elephant stall area. The appendix has additional details on the tracer gas testing.

Static Air Pressure Assessment

We evaluated the air pressure differences between the food preparation area and the elephant stall area in the phase 2 quarantine barn with a TSI® VelociCalc™ Plus Model 8386A electronic manometer. We put the manometer on the food preparation side of the barn with the manometer’s low-pressure line running through a previously installed tube that was part of a Ball-in-the-Wall® pressure indicator. We measured the air pressure differences with the elephant stall doors opened or closed and the exhaust fans on or off and with the doors to the food preparation area opened or closed.
Confidential Medical Interviews and Medical Record Review

We invited all 28 employees working during our final visit in September 2012 to participate in semistructured confidential interviews. During these interviews, we discussed their knowledge about TB disease, their work history and practices, their known exposures, their respiratory protection practices, and other related concerns. During these interviews, we educated employees about the signs and symptoms of TB disease. We also reviewed \textit{M. tuberculosis} screening and respiratory protection program records on all 30 individuals employed at the time of the visit.

Results and Discussion

Walk-through Surveys: General Observations

At the beginning of this evaluation, refuge managers believed that working in the phase 1 isolation barn presented the highest risk for exposure to \textit{M. tuberculosis} because this barn housed an elephant with active TB disease. During our evaluation, the refuge eventually housed one additional elephant also diagnosed with active TB disease in this barn.

A risk of exposure to \textit{M. tuberculosis} also existed in the phase 2 quarantine barn. During our initial walk-through survey, the elephants housed in this barn were trunk-wash culture negative and blood-test negative for \textit{M. tuberculosis}, but had known exposure to elephants with active TB disease. Over the course of this evaluation, two elephants in this barn developed active TB disease.

The risk of exposure to \textit{M. tuberculosis} in the New Asian and African barns was less clear. Years prior to this evaluation one elephant in the New Asian barn had previously received treatment for active TB disease. Another elephant in the African barn may have had a positive serological test indicating exposure to \textit{M. tuberculosis} prior to arrival at the refuge. It was unknown if other elephants in these barns had been exposed to \textit{M. tuberculosis}. Elephants were tested yearly for \textit{M. tuberculosis} by a trunk wash and culture.

Numerous dogs and cats live at the refuge and were seen around all of the barns. Dogs can become infected with \textit{M. tuberculosis} [Dannenberg 1978; Erwin et al. 2004; Turinelli et al. 2004; Sykes et al. 2007; Parsons et al. 2008]. However, the potential for zoonotic transmission of \textit{M. tuberculosis} between elephants and dogs, and dogs and humans is unclear. At least one report has indicated that dogs may act as a potential source of TB infection based upon necropsy of a dog that had an active TB infection [Parsons 2008]. Similar concerns have been raised about zoonotic transmission of \textit{Mycobacterium bovis} from dogs to humans on cattle farms. \textit{Mycobacterium bovis} is a bacterial species of the \textit{M. tuberculosis} complex and can be transmitted via respiratory aerosols in humans [CDC 2005a]. Reported signs and symptoms of active TB disease in dogs include chronic cough, weight loss, lethargy, and vomiting [Erwin et al. 2004; Turinelli 2004; Sykes et al. 2007]. It is unknown whether dogs with active TB disease can be asymptomatic. It has been reported that cats are highly resistant to \textit{M. tuberculosis} infection [Dannenberg 1978].
Personal Protective Equipment Review

**Phase 1 Isolation Barn**

All employees wore Tyvek® suits, nitrile gloves, muck boots, and an N95 filtering facepiece respirator during all work activities inside the barn and yard, including cleanup of hay and waste, elephant feeding, and administration of medication to the elephant with active TB disease. Before cleanup, the employees sprayed a disinfectant (Envirocide®) on the hay and waste using a small hand-held spray bottle. They let the disinfectant sit on the sprayed surfaces for at least 3 minutes, as recommended by the manufacturer, before water rinsing. During booster-assisted water cleaning (lower pressure than the high-pressure water cleaning system used in the phase 2 quarantine barn) of the elephant stalls, employees wore all of the PPE noted above plus a more protective 3M Air Mate™ loose-fitting powered air purifying respirator (PAPR) instead of the filtering facepiece respirator. The employees stored their PPE in a storage cabinet outside of the phase 1 isolation barn that was accessible to employees from the phase 1 isolation and phase 2 quarantine barns. Employees used a biohazard waste bin next to the storage shed to discard used disposable PPE. A contractor removed the biohazard waste from the site.

During our first visits in September and October 2010, we noticed that an electrical cord used in the barn was missing the grounding conductor (third prong) on the male plug end. We notified the refuge staff, and the electrical cord was removed from service. We also observed one employee working in this area with facial hair that interfered with the seal of the filtering facepiece respirator. We verbally reported these observations and recommendations to correct them during our site visit, as well as in an interim letter provided in March 2011.

**Phase 2 Quarantine Barn**

Employees working in this barn wore N95 filtering facepiece respirators. Observed work activities included food preparation, high pressure washing of the stall area, hand scrubbing of gates and bars in the stall area, and cleanout of the conveyor-assisted waste trench. Before cleanup, the employees sprayed Envirocide disinfectant on the hay and waste. Employees wore loose-fitting PAPRs during potential aerosol-generating activities, such as high-pressure washing and hand scrubbing in the barn, or high-pressure washing of the conveyor-assisted waste trench. Employees also wore nitrile gloves and muck boots during the potential aerosol-generating activities, but not Tyvek suits.

**New Asian and African Barns**

Employees did not use engineering controls or PPE to prevent exposures to *M. tuberculosis* in these areas. This may present a risk of exposure if an elephant has unrecognized TB disease.

**Review of Clothing and Boot Written Protocols**

We provided clothing recommendations for working in phase 2 quarantine barn areas in our letter dated March 31, 2011. To reduce contamination of skin and clothing, we recommended employees wear Tyvek suits during aerosol generating activities where clothing can become
soiled or wet. This protocol was already in place for employees working in the phase 1 isolation barn during our October 2010 visit. We agreed with the refuge’s updated policy to wear uniforms during the workday and to launder these uniforms at the facility. However, in the phase 1 isolation and phase 2 elephant stall areas or when working within 25 feet of any elephant, wearing Tyvek suits over the work uniform during aerosol generating activities would prevent the contamination of work clothes and provide additional protection for employees. Other types of overalls, including cotton, could be substituted for Tyvek, though these alternatives may be less water resistant and require more frequent changing to ensure comfort.

At the time of our September 2012 visit, all of the written protocols required employees entering any elephant barns to change into, or arrive in, a clean work uniform, and to wear this uniform for the entire time at the barn area. Employees were also required to change into a new work uniform if their uniform became “excessively soiled” with respiratory discharge or feces during the workday. When they left the barn area, employees were required to change out of the uniform and back into street clothes. Dirty uniforms were left at the barn and laundered onsite by staff.

Separate sets of boots were stored at each barn. Before entering the barns, employees put on their barn specific boots and removed them before leaving the barn areas. One set of boots was required when working in the phase 1 isolation barn, phase 1 habitat, and phase 2 habitat. Another set of boots was required for working in the phase 2 quarantine barn. Employees not caring for the elephants (maintenance employees, other staff, and contractors) were provided boots or boot covers when entering any elephant stalls. These employees could wear street shoes in the caregiver areas at the New Asian or African barns.

It is important to note that prolonged use of PPE by employees working in the barn areas during the summer months could increase the possibility of heat stress.

**Ventilation Smoke Assessment**

**Phase 1 Isolation Barn**

The exhaust fans did not remove theatrical smoke from the barn in any of the test conditions (summer, natural ventilation and summer, and winter), regardless of whether the HEPA filter was used. We found that the exhaust fan above the elephant stall on the north side of the building was not working. We found air leaking around the HEPA filter and recirculating in the roof-mounted cupola. Causes for this air recirculation include (1) using vane axial exhaust fans in the cupolas that were incapable of providing the static pressure required to pull the exhaust air through the HEPA filter and (2) placing HEPA filters between the ceiling grates and vane axial (fans prior to our evaluation) that reduced the airflow.

**Phase 2 Quarantine Barn**

We found that operating the exhaust fans prevented air from flowing from the stall area into the keeper areas. This happened regardless of whether the three elephant doors and double man door to the outside were open or closed. However, according to refuge managers,
they only operated the exhaust fans during pressure washing in warm weather. They did not operate the exhaust fans during cold weather because of excessive heat loss. Using ventilation smoke in the fans-off scenario, we observed air migrating through the bottom of the previously sealed double doorway separating the second floor rest area and the observation deck overlooking the elephant stalls.

**New Asian and African Barns**

The New Asian barn’s exhaust fan design and performance were similar to the phase 2 quarantine barn. In the New Asian barn, theatrical smoke generated in the elephant stall area drifted into the keeper areas of the barn when the exhaust fans were off. However, our attempts to observe air flow in the barn under varying conditions (barn door open/closed, and exhaust fan on/off) were complicated by wind, making it difficult to compare our observations. Generally, we observed that during simulated winter conditions (seven of the nine barn doors closed) and with all of the exhaust fans turned on, air did not flow from the elephant stalls into the keeper areas. However, like the phase 2 quarantine barn, these exhaust fans were reported to be rarely used in winter because of excessive heat loss.

The African barn did not have exhaust fans or any other engineering controls to prevent shared air between elephant stall and keeper areas.

**Tracer Gas Ventilation Assessment**

**Phase 2 Quarantine Barn**

We made our tracer gas measurements on April 27, 2012, after the refuge made the following engineering changes on the basis of our recommendations.

- Changed supply ventilation ductwork to redirect air from the second floor administrative space into the food preparation area.
- Sealed openings in the ceiling and interior walls separating the food preparation area and elephant stalls.
All of the sulfur hexafluoride measurements made during our September 2012 visit were background-corrected (see appendix). We then divided the average tracer gas concentration on the elephant side of the phase 2 quarantine barn sealed wall by the average tracer gas concentration in each test area on the administrative side of the sealed wall to calculate a protection factor (Table 3). In this instance the protection factor also includes the protection offered by the sealed wall as well as the positive pressure maintained in the administrative side compared to the elephant stalls. Positive pressure observed in this area means that air flowed from the administrative side into the elephant stalls, a desirable ventilation design.

Table 3. Tracer gas testing and calculated protection factors

<table>
<thead>
<tr>
<th>Date</th>
<th>Sulfur hexafluoride concentration, in parts per million</th>
<th>PF†</th>
<th>Second floor admin</th>
<th>PF</th>
<th>Entry/exit area</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/11/12</td>
<td>21</td>
<td>&lt; 0.01*</td>
<td>2,100</td>
<td>&lt; 0.01*</td>
<td>2,100</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>9/12/12</td>
<td>28</td>
<td>&lt; 0.01*</td>
<td>2,800</td>
<td>&lt; 0.01*</td>
<td>2,800</td>
<td>0.026</td>
</tr>
</tbody>
</table>

PF = Calculated protection factor
*Concentration was less than the limit of detection of 0.01 parts per million.
†PF was calculated by dividing the air concentration measured in the elephant stall by the air concentration measured in the tested areas.
As shown in Table 3, the calculated protection factors for the food preparation and second floor administrative areas exceeded 2,000 for both test days. In the entry/exit area, the calculated protection factor on September 12, 2012, was approximately 50% lower than the protection factor calculated for the day before. This was likely from employee traffic during the September 12, 2012, test. This result is consistent with other studies that have shown anteroom contamination resulting from employee movement [Hayden et al. 2007; Johnson et al. 2009; Hyttinen et al. 2011]. As a note, the Centers for Disease Control and Prevention (CDC) defines an anteroom as “a small room leading from a corridor into an airborne infection isolation room. An anteroom is separated from both the airborne infection isolation room and the corridor by doors. An anteroom can act as an airlock, preventing the escape of contaminants from the airborne infection isolation room into the corridor” [CDC 2005]. We estimated the effective dilution ventilation rate for this entry/exit area to be 30 air changes per hour (ACH). This is a desirable ventilation design because it exceeds the 10 ACH recommended by CDC for anterooms attached to airborne infection isolation rooms used for tuberculosis prevention [CDC 2005b].

In comparison, all of the calculated protection factors for these barn areas exceeded protection factors assigned to air-purifying respirators. For example, the assigned protection factor for a properly fitted and worn filtering facepiece respirator (e.g., an N95 respirator) is 10, and for a loose-fitting PAPR it is 25 [29 CFR 1910.134]. OSHA defines an assigned protection factor as “a workplace level of respiratory protection that a respirator or class of respirators is expected to provide to employees when the employer implements a continuing, effective respiratory protection program” [29 CFR 1910.134].

**Tracer Gas Concentration Decay Analyses – Phase 1 Isolation and Phase 2 Quarantine Barns**

On the basis of how quickly the tracer gas concentration in the barns dissipated, we calculated the average effective ventilation rates, expressed in ACH (Table 4). The effective ventilation rate represents the ventilation system’s ability to remove an airborne contaminant via dilution or direct capture and exhaust. Thus, the higher the effective ACH, the better the ventilation system’s protective performance.

<table>
<thead>
<tr>
<th>Test description</th>
<th>Average effective ventilation rate across two locations (ACH)</th>
<th>Time to achieve 90% reduction in tracer gas (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1 isolation barn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exhaust fans on, all doors closed</td>
<td>2.6</td>
<td>53</td>
</tr>
<tr>
<td>Exhaust fans on, all doors open</td>
<td>14.3</td>
<td>10</td>
</tr>
<tr>
<td>Covered area – natural ventilation only</td>
<td>24.5</td>
<td>6</td>
</tr>
<tr>
<td>Phase 2 quarantine barn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exhaust fans on, all barn doors open</td>
<td>14.8</td>
<td>9</td>
</tr>
<tr>
<td>Exhaust fans on, barn, doors at opposite</td>
<td>25.4</td>
<td>5</td>
</tr>
<tr>
<td>end of fans open</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ingress/egress pass-through area</td>
<td>30</td>
<td>&lt; 6</td>
</tr>
</tbody>
</table>
In the phase 1 isolation barn, the average effective ventilation rate was lowest when the exhaust fans were on and all barn doors closed (Table 4). The effective ventilation rate increased when the exhaust fans operated with all of the barns doors open. The covered area, which had no exhaust fans but was open to the outside, had a higher effective ventilation rate than the barn, likely the result of concentration dilution under the ambient environmental conditions that day.

In the phase 2 quarantine barn, we measured the highest average effective ventilation rate when the exhaust fans were on and the two elephant doors at the opposite end of the barn were open (Table 4). We believe that this higher effective ventilation rate was due to a wind-tunnel effect, where the exhaust fans pulled make-up air into the open barn doors at the far end of the barn. This make-up air then swept through the barn before exhausting through the wall fans.

There are no established ventilation control guidelines for barns for elephants or other animals with airborne infectious diseases. However, there are ventilation guidelines for airborne infection isolation rooms in human healthcare settings that are used to house patients with diseases that can be spread by infectious aerosols including active TB disease. The CDC guidelines recommend that airborne infection isolation rooms provide at least 12 ACH [CDC 2005b]. We found that the ventilation systems in the phase 1 isolation and phase 2 quarantine barns exceeded this guideline when the doors were open. This should be the case regardless of the time of year.

We also calculated the time required to reduce the tracer gas concentration by 90% of its original concentration (Table 4). In the phase 1 isolation barn, tracer gas concentration was reduced by 90% in 53 minutes with the doors closed, a typical arrangement used by the staff to purge the area. With the exhaust fans on and doors open, the tracer gas concentration in the phase 1 isolation barn was reduced five times more quickly (90% reduction in 10 minutes). In the outdoor covered area next to the phase 1 isolation barn, the tracer gas concentration was reduced by 90% in 6 minutes. In the phase 2 quarantine barn, the tracer gas concentration was reduced by 90% in 5 minutes, with the wall-mounted exhaust fans on and two doors on the opposite end of the barn open, creating a wind-tunnel effect.

Although ambient environmental conditions, such as wind speed, can affect the calculated 90% reduction times, these data suggest preferred work practices that would increase the average effective ventilation rate, thus reducing potential employee exposures to infectious aerosols. For example, there was a smaller increase in the effective ventilation rate increase when operating the exhaust fans alone in the phase 1 isolation barn compared to opening the doors in combination with operating the exhaust fans (Table 4). However, the all-doors-open approach was not the most effective approach in the phase 2 quarantine barn (Table 4) because the wind-tunnel effect created by just opening the doors on the opposite wall from the operating fans was the most effective arrangement to reduce tracer gas concentrations. In the ingress/egress pass-through area in the phase 2 quarantine barn, the effective ventilation rate of 30 ACH exceeded the 10 ACH recommended by CDC for anterooms attached to airborne infection isolation rooms [CDC 2005b], and the tracer gas concentration was reduced by 90% in less than 6 minutes (Table 4).
Air Flow Tests

The static air pressure measurements taken in the phase 2 quarantine barn are shown in Table 5. There are no guidelines for elephant barns, but the CDC recommends a minimum positive pressure differential of 0.01 inches of water gauge between areas adjacent to TB patient rooms (e.g., hallways or anteroom) and TB patient rooms [CDC 2005b]. Pressure differential is defined as the difference in atmospheric pressure between two different areas. A negative pressure differential indicates that the atmospheric pressure measured in one area is lower than the atmospheric pressure measured in an adjacent area. Using these criteria, all of the evaluated test conditions except condition 6 met the minimum pressure differential criterion of 0.01 inches of water gauge (Table 5). In condition 6, there were no pressure differences because both areas were open to the outdoors. This finding emphasizes the importance of keeping the food preparation doors closed so that this area remains under positive pressure relative to the elephant stalls. When the food preparation area is maintained under positive pressure, any pressure driven air flow leaks between the two areas will occur from the food preparation area into the stalls. This finding also suggests that an anteroom between the food preparation areas and the outdoors could offer an added level of protection. This could be established by constructing an entryway with two sets of entry doors in series that could help maintain the desirable pressure difference, as long as only one set of doors is open at a time.

Table 5. Static pressure differences between food preparation and elephant stall areas

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pressure difference, in inches of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Food preparation doors closed, barn doors closed, barn exhaust fans on, fan doors open</td>
<td>+0.26</td>
</tr>
<tr>
<td>2. Food preparation doors closed, barn doors closed, barn exhaust fans off, fan doors open</td>
<td>+0.035</td>
</tr>
<tr>
<td>3. Food preparation doors closed, barn doors closed, barn exhaust fans off, fan doors closed</td>
<td>+0.024</td>
</tr>
<tr>
<td>4. Food preparation doors closed, barn doors open, barn exhaust fans on, fan doors open</td>
<td>+0.030</td>
</tr>
<tr>
<td>5. Food preparation doors closed, barn doors open, barn exhaust fans off, fan doors open</td>
<td>+0.030</td>
</tr>
<tr>
<td>6. One food preparation door open to outdoors, barn doors open, barn exhaust fans off, fan doors open</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Confidential Medical Interviews and Medical Record Review

During the final visit in September 2012, we interviewed 27 of the 28 employees working during that time either in person or by phone. The median age of the 27 interviewed employees was 28 years, with a range 19 to 65 years; 21 were female.

The median amount of time the 27 interviewed employees reported working at the center was 2.5 years, with a range of 6 weeks to 10 years. The median hours worked per week was 40 hours, with a range of 30 to 55 hours. Other work characteristics of the 27 interviewed employees are shown in Table 6.

Table 6. Work characteristics of interviewed employees

<table>
<thead>
<tr>
<th>Work characteristic</th>
<th>No. employees (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Job category</strong></td>
<td></td>
</tr>
<tr>
<td>Caregiver</td>
<td>11 (41)</td>
</tr>
<tr>
<td>Administrative</td>
<td>9 (33)</td>
</tr>
<tr>
<td>Senior management*</td>
<td>5 (19)</td>
</tr>
<tr>
<td>Maintenance</td>
<td>2 (7)</td>
</tr>
<tr>
<td><strong>Primary job location</strong></td>
<td></td>
</tr>
<tr>
<td>Welcome center</td>
<td>8 (30)</td>
</tr>
<tr>
<td>Quarantine barn area</td>
<td>7 (26)</td>
</tr>
<tr>
<td>New Asian and/or African barn</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Darbytown office</td>
<td>4 (15)</td>
</tr>
<tr>
<td>Maintenance shop</td>
<td>2 (7)</td>
</tr>
<tr>
<td><strong>Work activities</strong></td>
<td></td>
</tr>
<tr>
<td>Prepare feed</td>
<td>13 (46)</td>
</tr>
<tr>
<td>Feed elephants</td>
<td>13 (46)</td>
</tr>
<tr>
<td>Administer medications to elephants</td>
<td>11 (41)</td>
</tr>
<tr>
<td>Participate in elephant trunk washes</td>
<td>11 (41)</td>
</tr>
<tr>
<td>Sweep or shovel hay, sawdust, and excrement from stalls</td>
<td>11 (41)</td>
</tr>
<tr>
<td>Pressure/booster wash barns walls and floors</td>
<td>11 (41)</td>
</tr>
</tbody>
</table>

*Senior management includes veterinary staff

Fifteen (54%) interviewed employees reported having close contact with elephants (defined as touching or being close enough to touch) at work. Eleven (41%) interviewed employees reported having close contact with at least one elephant known to have active TB disease at work.

Twenty-three (85%) interviewed employees reported having attended general training on TB during their employment. Thirteen (48%) employees correctly identified at least two symptoms of active TB disease in humans; 21 (78%) correctly identified at least one symptom.
Our record review revealed that 27 (90%) of the 30 current employees were medically cleared to wear a respirator, though dates of medical clearance were missing in the records of 13 of these employees. Twenty-three (77%) of the 30 current employees underwent respirator training and fit testing since they started work at the center. However, only 16 (70%) employees had undergone respirator training and fit testing within the previous year.

Seventeen interviewed employees reported spending time in the phase 2 quarantine barn area, an area where respiratory protection is required. Of the seventeen interviewed employees, four (24%) had not undergone respirator training and fit testing within the previous year. In addition, of the 11 employees who reported having close contact with at least one elephant known to have active TB disease, three had not undergone respirator training and fit-testing for N95 filtering facepiece respirators within the previous year.

All 16 employees who reported coming within 25 feet of the quarantine barn or within 25 feet of any elephant residing in the quarantine barn reported always using an N95 filtering facepiece respirator or a PAPR. All six employees who reported sweeping out the New Asian and African barns reported always wearing an N95 respirator or PAPR when doing so. All six employees who reported booster cleaning the quarantine barn, and all seven employees who reported booster or pressure cleaning the New Asian or African barns reported always using a PAPR when doing so. However, of the 12 employees who reported training for or performing trunk washes, five reported always using a PAPR, five reported always using an N95 filtering facepiece respirator, one reported always using either a PAPR or N95 respirator, and one reported using an N95 filtering facepiece respirator most of the time.

According to employee records and interviews, three (10%) of the 30 current employees reported a history of a positive tuberculin skin test (TST) or latent TB infection; one employee had a TST conversion that occurred prior to employment at the center. Two completed the recommended 9 months of isoniazid therapy. Only one of these three employees reported having an evaluation for symptoms of active TB disease in the previous year. The remaining 24 employees had histories of negative TSTs. Of these, one employee had not had a TST in the previous year; the other 23 employees had TSTs in the previous 4 months.

Our interviews revealed that exposure to the elephants with active TB disease is limited to the essential employees for that area, and employees reported excellent compliance with respiratory protection recommendations. Nevertheless, our investigation revealed the following gaps in the implementation of administrative and respiratory protection controls.

- From our interviews we found that only one of three employees with a history of latent TB infection reported having an evaluation for symptoms of active TB disease in the previous year. Because these employees do not undergo further TST testing, it is important that they undergo this clinical evaluation.
- Employees working in areas outside of the quarantine barn were confused as what type of respiratory protection was necessary for participation in trunk washes.
- Our record review revealed some employees working in the quarantine barn area, including some who reported having close contact with elephants known to have active TB disease, had not undergone the recommended annual respirator training and fit
testing within the previous year. Annual fit testing is important to ensure proper fit of the respirator and to minimize the risk for being infected with TB.

Because none of the elephants with active TB disease were undergoing treatment at the time of our third visit, compliance with these other controls remained of the utmost importance.

Conclusions

A health hazard from exposure to \textit{M. tuberculosis} existed at this elephant refuge. The employee TB screening program needed improvement. Our evaluation identified deficiencies in engineering controls, administrative controls, work practices, and PPE use. However, the engineering changes made to the barns during this evaluation resulted in improved ventilation and reduced or eliminated air movement from the elephant stalls (where air potentially contaminated with \textit{M. tuberculosis} may be present) into food preparation and administrative areas. Identification of elephants with active TB disease and antimycobacterial treatment of those elephants remain of the utmost importance in reducing the risk of tuberculosis transmission to employees.

Recommendations

On the basis of our findings, we recommend the actions listed below to further reduce the risk of transmission of \textit{M. tuberculosis} from elephants to employees. We encourage managers and employees to discuss our recommendations and develop an action plan. Often those employees involved in the work are best suited to set priorities and assess the feasibility of our recommendations.

Our recommendations are based on an approach known as the hierarchy of controls. This approach groups actions by their likely effectiveness in reducing or removing hazards including elimination or substitution, administrative controls, engineering controls, and PPE. Unlike other occupational exposures, for TB, environmental controls, such as engineering controls, are considered a second line of defense in the TB infection control program after administrative controls [CDC 2005b].

Elimination and Substitution

Eliminating or substituting hazardous processes or materials reduces hazards and protects employees more effectively than other approaches. Prevention through design, considering elimination or substitution when designing or developing a project, reduces the need for additional controls in the future.

1. Consider euthanasia of elephants with active TB disease, which would lower the risk of zoonotic transmission from elephants to employees. However, we are sensitive to the ramifications of euthanasia and understand that USAHA guidelines [2010], adopted by USDA [2012], indicate that treatment is the preferred option for elephants that are culture positive. It is important to note that USAHA [2010, 2012] indicates that euthanasia “may be considered for those animals that are showing clinical signs
considered to be poor candidates for treatment or for other factors based on the clinician’s discretion.”

**Administrative Controls**

Administrative controls refer to employer-dictated work practices and policies to reduce or prevent hazardous exposures. Their effectiveness depends on employer commitment and employee acceptance. Regular monitoring and reinforcement are necessary to ensure that policies and procedures are followed consistently.

**All Areas**

1. Treat all elephants with active TB disease as soon as possible to reduce exposures to employees.

2. Provide general TB training during working hours to all employees on hire and annually thereafter to ensure a thorough understanding of the disease, its symptoms, its transmission, and ways to prevent it. General training and education materials can be found on the CDC TB website at [http://www.cdc.gov/tb/](http://www.cdc.gov/tb/).

3. Continue at least annual TST placement for employees with negative TST histories.

4. Ensure that employees with new TST conversions receive a prompt medical evaluation.

5. Ensure that employees with a previous history of latent TB infection undergo a clinical evaluation for symptoms of TB disease annually.

6. Consider the use of two sets of disposable gloves in both barns during work activities that result in soiling of hands with elephant waste. When removing PPE after these tasks are finished, carefully remove the outer layer (heavily soiled) pair of gloves first. Remove remaining PPE with the inner layer of gloves. This will minimize further contamination when removing other PPE.

7. Wash hands after removing PPE. CDC recommends that the optimal method is to wash with soap and warm water for 20 seconds. Additional information on hand washing can be found on the following CDC webpage at [http://www.cdc.gov/handwashing/](http://www.cdc.gov/handwashing/).

8. Create a heat stress prevention program. Employees working outdoors at the refuge may have risk for developing heat illness-related symptoms during warmer months. In addition, employees working in the phase 1 isolation and phase 2 quarantine barns are at higher risk of heat stress because of PPE usage in these areas. Some important components of a heat stress program include:

   a. Requesting employees discuss their hot work with their physician. The physician can let them know if they have a health condition or are taking medication that may increase their risk of heat-related illness.

   b. Training employees to know the dangers and how to protect themselves when working in extreme heat.

   c. Allowing employees the time to get used to the heat (acclimatize) by exposing them for progressively longer periods to hot work. This is needed for new
employees, returning employees, and any time that temperatures increase.

d. Providing employees a cool area and water for breaks, and encouraging employees to take breaks.

e. Encouraging employees to report any heat illness-related symptoms and signs.

f. Keeping systematic records of employee reports of heat-related illnesses.

g. Teaching employees to monitor themselves and others for signs and symptoms of heat-related illness. Employers/supervisors should also participate in monitoring, especially if someone does not have a buddy with them.

Additional information on the symptoms and first aid for heat-related illnesses and recommendations to reduce risk are available at http://www.cdc.gov/niosh/topics/heatstress/.

9. Ensure that all electrical extension cords are in safe working order. Per the OSHA electrical standard (29 CFR 1910.334), extension cords should be “visually inspected before use on any shift for external defects (such as loose parts, deformed and missing pins, or damage to outer jacket or insulation) and for evidence of possible internal damage (such as pinched or crushed outer jacket).” Also per OSHA, do not remove grounding conductors from electrical cords, and remove and repair or discard any damaged electrical cords.

10. Keep dogs away from elephants with known or suspected TB disease. Regularly check dogs at the refuge for signs and symptoms of potential active TB disease. Signs and symptoms of active TB reported in dogs include chronic cough, weight loss, lethargy, and vomiting. If you suspect active TB disease, a veterinarian should evaluate the dog.

**Phase 2 Quarantine Barn**

1. Perform a daily check of the Ball-in-the-Wall pressure indicator when elephants with known or suspected TB disease are present to ensure that the food preparation area is under positive pressure relative to the elephant stall area.

2. Keep the doors to the food preparation area closed except when exiting this area. Employee traffic in and out of the food preparation area should be limited during times when the elephants are inside the elephant stall area.

**Engineering Controls**

Engineering controls reduce employees’ exposures by removing the hazard from the process or by placing a barrier between the hazard and the employee. Engineering controls protect employees effectively without placing primary responsibility of implementation on the employee.

**Phase 1 Isolation Barn**

1. Maintain this barn as open as possible to the extent allowed by environmental conditions. On a day-to-day basis, keep the windows and doors open as much as possible to increase the effective ventilation rate. This will allow wind and convective currents to dissipate potentially infectious aerosols into the atmosphere where they will become quickly diluted and eventually deactivated by natural ultraviolet light.
2. Purge the air from the elephant stall area by turning on the exhaust fans and opening all doors before employees enter this area. The exhaust fans should operate for at least 15 minutes after the barn has been occupied by elephants with known or suspected TB disease to reduce concentrations of potentially-infectious aerosols before employees enter this area. Additionally, the exhaust fans should remain operating in the following circumstances: (a) during and after potential aerosol generating activities, and (b) when elephants and employees are occupying the elephant stall area. Employees should wear N95 filtering facepiece or greater level of respiratory protection at all times when they are in the isolation barn.

3. Move the open and close door switches in the elephant barn to outside the barn.

**Phase 2 Quarantine Barn**

1. Install self-closing exterior doors in the food preparation area. Alternatively, consider installing revolving doors or double-wall/double-door vestibules at the entry to maintain this area under positive pressure relative to adjacent areas.

2. Use the wind-tunnel ventilation approach by turning on the exhaust fans and opening the two elephant doors on the opposite wall across from the exhaust fans to purge the air from the elephant stall regardless of whether or not elephants are present in the barn. After it has been occupied by elephants with known or suspected TB disease, the barn air should be purged for at least 15 minutes to reduce airborne concentrations of potentially infectious aerosols before employees enter the elephant stall area. Once the purge period is complete and employees enter the elephant stall area, the wind-tunnel ventilation strategy should continue during the following conditions: (a) during and after potential aerosol generating activities and (b) when elephants and employees are occupying the elephant stall area. Additionally, employees should use the wind tunnel ventilation strategy to their advantage and place themselves “upwind” of elephants and aerosol generating activity whenever possible. Employees should wear N95 filtering facepiece or greater level of respiratory protection even after purging.

**Personal Protective Equipment**

PPE is the least effective means for controlling hazardous exposures. Proper use of PPE requires a comprehensive program and a high level of employee involvement and commitment. The right PPE must be chosen for each hazard. Supporting programs such as training, change-out schedules, and medical assessment may be needed. Personal protective equipment should not be the sole method for controlling hazardous exposures. Rather, PPE should be used until effective engineering and administrative controls are in place.

**All Areas**

1. Review and update the written respiratory protection program. Appoint a program administrator who is trained on the requirements in the OSHA respiratory protection standard [29 CFR 1910.134]. All employees who may need to wear respirators should be medically cleared, and dates of clearance should be documented. In addition, these employees should receive annual training and undergo fit testing annually as required.
in the OSHA respiratory protection standard [29 CFR 1910.134].

2. Clarify in the TB control policy whether N95 filtering facepiece respirators or PAPRs are to be worn by employees participating in trunk washes and other aerosol generating procedures. Protection greater than an N95 filtering facepiece respirator (e.g., a full-facepiece elastomeric respirator or PAPR) should be considered for aerosol generating procedures such as high-pressure washing in the barn. Clarify activities that should occur in the donning and doffing areas outlined in the clothing and boot protocols. Used or soiled uniforms should not be worn into the donning area. Additionally, employees should not be allowed to wear soiled uniforms into the break area near the phase 2 quarantine barn.

**Phase 1 Isolation Barn and Phase 2 Quarantine Barn**

1. Wear respiratory protection (N95 filtering facepiece respirator or greater) when working within 25 feet of an elephant with known or suspected TB disease or within the elephant stall area, regardless of whether the ventilation systems are functioning. It is important to note that the 25-foot distance is recommended in the absence of other data; no evidence is available to define a safe distance from a TB-infected elephant.

2. Wear disposable coveralls such as Tyvek on top of the work uniform during aerosol generating activities to reduce contamination of skin and clothing.

3. Post signs at the entrances to the barn areas reminding entering employees of the personal protective equipment requirements.

**Phase 2 Quarantine Barn**

1. Respiratory protection is not required for employees working in the food preparation area when this location is under positive pressure relative to the elephant stalls. This means that air flows from the food preparation area into the elephant stalls.
Appendix: Tracer Gas Testing

Phase 2 Quarantine Barn Containment Tests

We used one Brüel and Kjær (B&K) Model 1302 Photoacoustic Multigas monitor and one LumaSense™ Technologies Innova 1312 Photoacoustic Multigas monitor (Innova) to measure tracer gas concentrations on the elephant side of the sealed wall. We mounted the B&K monitor on a portable cart and sampled at human breathing zone height, about 4 feet above the floor. We sampled along the walking corridor in the elephant stall area, adjacent to the sealed wall separating the elephant stall area from the food preparation area. All sulfur hexafluoride concentrations were background-corrected using the mean monitor response over the five readings prior to the release of tracer gas. If this correction resulted in results that were less than zero, we reset the results to zero to avoid having negative data values.

We placed the Innova monitor about 4 feet above the second floor observation deck, an area employees no longer used because the access door had been sealed. For the first tracer gas release, three Thermo Scientific™ MIRAN SapphIRe Portable Ambient Analyzers (MIRAN) collected tracer gas measurements on the administrative side of the sealed separation wall. The areas monitored included the second floor administrative area adjacent to the observation window, the food preparation area (first floor), and the ingress/egress pass-through into the elephant stall area (first floor).

For the second tracer gas release, we placed a fourth MIRAN analyzer at the ingress/egress pass-through area because of observed monitor response abnormalities during the first test. The B&K and MIRAN monitors remained in their same sampling positions for the tracer gas concentration decay tests that followed each containment test. During the tracer gas containment testing, the ventilation system serving the food preparation side of the barn was on, and the exhaust fans in the elephant stall were off.

Tracer Gas Concentration Decay Analyses

In the phase 1 isolation barn, we conducted two sequential tracer gas concentration decay analyses in the indoor elephant stall area and one in the adjacent outdoor elephant contact area. The outdoor contact area had an overhead roof and an opaque plastic sidewall. The contact area had locking gates and bars that allowed employees to access the elephants safely. We used the B&K and the Innova monitors for these analyses. Initially, we released the tracer gas inside the barn with the overhead and man doors closed and exhaust fans turned off. After the tracer gas was released and mixed with a fan and leaf blower, the exhaust fans were turned on for about 17 minutes, and tracer gas levels were recorded. We then opened all doors in the barn and continued to record tracer gas levels for an additional 12 minutes. In the covered contact area we released the tracer gas and allowed natural dilution and mixing to occur for 2 minutes. After that time, we monitored the tracer gas levels for approximately 8 minutes.

A separate tracer gas concentration decay analysis was conducted in the phase 2 quarantine barn immediately following each of the tracer gas containment tests on September 11 and 12, 2012. In the first decay test, the two exhaust fans on the side of the phase 2 quarantine
barn were turned on, and all barn doors were opened. In the second test, both exhaust fans were turned on, the two elephant doors at the opposite end of the barn were opened, and the remaining doors were left closed. We used the B&K and the Ínnova monitors to measure the concentration of tracer gas over time during each of the test conditions.
References


CDC (Centers for Disease Control and Prevention) [2005b]. Guidelines for preventing transmission of Mycobacterium tuberculosis in health-care settings. MMWR 54(RR-17):1–141.


Keywords: North American Industry Classification System 115210 (Support Activities for Animal Production), Tennessee, TB, tuberculosis, *Mycobacterium tuberculosis*, ventilation, tracer gas, elephant, refuge, PPE, zoonosis
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