Virulence of fungal spores determined by tracheal inoculation of goats following inhalation of aerosolized sterile feedyard dust

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Objective—To compare the virulence of spores of 7 fungi by tracheal inoculation of goats following exposure of goats to an aerosol of sterilized feedyard dust.

Animals—54 weanling Boer-Spanish goats.

Procedure—A prospective randomized controlled study was conducted. There were 7 fungal treatment groups, a tent control group, and a pen control group (n = 6 goats/group). Goats in the 7 treatment and tent control groups were exposed to autoclaved aerosolized feedyard dust for 4 hours in a specially constructed tent. Goats in the 7 treatment groups were then inoculated intratracheally with 30 mL of a fungal spore preparation, whereas tent control goats were intratracheally inoculated with 30 mL of physiologic saline (0.9% NaCl) solution. These treatments were repeated each week for 6 weeks.

Results—Severity of pathologic changes differed significantly among the 7 fungal treatment groups as determined on the basis of gross atelectatic and consolidated lung lesions and histologic lesions of the lungs. Descending order for severity of lesions was *Mucor ramosissimus, Trichoderma viride, Chaetomium globosum, Stachybotrys chartarum, Aspergillus fumigatus, Penicillium chrysogenum,* and *Monotospora lanuginosa*. *Trichoderma viride* spores were the most invasive and were isolated from the bronchial lymph nodes and thoracic fluid of all 6 goats administered this organism. Spores were observed-histologically in lung tissues harvested 72 hours after inoculation from all treatment groups.

Conclusions and Clinical Relevance—4 of 7 fungal spore types induced significantly larger lung lesions, compared with those induced by the other 3 spore types or those evident in control goats. (*Am J Vet Res* 2005;66:615–622)

The most toxic part of feedyard dust is endotoxin and not viable bacteria or fungi.[1] However, many genera of fungi are found in feedyard dust,[2] some of which have the capability to induce infections or toxic reactions in animals under certain conditions. The respiratory tract of healthy animals has an enormous capacity to rapidly clear inspired dust particles and microbes through various innate defensive mechanisms, such as the mucocillary escalator process,[3] surface liquid of the respiratory system,[4] filtering hairs in the nares,[5] convoluted sinuses, and the reticuloendothelial system.[6] Dust or microbial particles in the range of 8 to 10 µm lodge in the nasal passages or sinuses, smaller particles (5 to 6 µm in diameter) are removed through action of the ciliated proximal portion of the respiratory tract, and the smallest particles (≤ 3 µm in diameter) may lodge in the alveoli at a point distal to the reach of the mucocillary escalator mechanism.[7,8] The smallest particles that lodge in the alveoli may be removed and digested by phagocytes, mobile phagocytes may engulf them and move them to the mucocillary escalator apparatus,[9] or mobile phagocytes in the lymph may move them to the regional thoracic lymph nodes for processing.[10] When a microbial pathogen manages to evade the innate defensive systems by surviving in macrophages or evading extracellular phagocytosis, the adaptive immune system will defend the lungs against such microbes.[11] Major risk factors for dissemination of *Aspergillus* organisms that cause infections are quantitative or qualitative disorders of innate phagocyte function.[12]

Small dust particles may physically fill alveolar macrophages or phagocytes deep in the lungs. Alveolar macrophages may be overloaded and impaired, which has been documented in all species of animals tested.[13] However, information concerning dust overload in nonrodent species is urgently needed[14] because variation among species may impact differences in dust overloading of macrophages. The probable cause of impairment is the suppressed movement of overloaded alveolar macrophages away from the dust-laden area.[13,15]

When fungal spores escape the innate immune system, then T- and B-cell acquired immunity develops.[11] It is conceivable that fungal spores could infect the lungs or release immunosuppressive mycotoxins[16,17] that may damage delicate lung tissues. The objective of the study reported here was to compare the virulence of 7 fungal spore types administered weekly to goats via intratracheal inoculations for 6 weeks. Prior to each tracheal inoculation, goats were confined in a tent and exposed to aerosolized sterile feedyard dust for 4 hours. The hypothesis tested was that spores from various fungal genera differed in pathogenicity in lungs of goats exposed to feedyard dust.

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Materials and Methods

Animals—Fifty-four Boer-Spanish crossbred weanling goats were used in the study. Goats were 10 to 16 weeks old. They were housed in a 3-sided barn in 9 pens (6 goats/pen); each pen was 7,420 cm^2. Goats were administered medications to eliminate internal parasites[a] and coccidia.[b] Goats were limit-fed a commercial pelletized ration (44% grain concentrate, 20% alfalfa hay, 30% cottonseed hulls and meal, 5% molasses, vitamins A and E, and trace minerals); water was available ad libitum. The experimental protocol was approved by a regional animal care and use committee.

Experimental design—Goats were allowed to acclimate to their environment for 2 weeks. Each pen of goats was then randomly allotted to 7 treatment groups and 2 control groups. The tent control group was used to evaluate the effect of being confined in a tent filled with aerosolized dust for 4 hours followed by intratracheal administration of 30 mL of saline (0.9% NaCl) solution. Goats in the pen control group were not manipulated (ie, not confined in the dust-filled tent and did not receive intratracheal inoculations).

Administration of aerosolized dust to goats—Goats in the 7 treatment groups and tent control group were exposed to aerosolized feedyard dust. The feedyard dust preparation has been characterized and described elsewhere.[1] Size of dust particles was determined by dry dispersion. Three 1-g samples were each passed through an instrument[c] to determine distribution of mean particle size. The dust was autoclaved[d] for 15 minutes at 121°C and 6.9 kPa of pressure to kill microbes (bacteria, fungi, and fungal spores) typically found in feedyard dust.[2] Cultures of autoclaved dust did not yield viable colonies of bacteria or fungi.

The aerosolization process has been described in detail elsewhere.[1,18] Briefly, dust was administered to goats contained in a semi-airtight tent.[e] A 1,500-g aliquot of sterilized feedyard dust was placed in a hopper[f] that augered the dust into a funnel, which led to a jet mill.[g] Dissemination of dust by the jet mill was augmented with air produced by a gasoline-powered portable air compressor[h] and air from a large blower motor.[i] Throughout a period of 4 hours, goats were exposed to 950 g of aerosolized dust inside the tent. The 4-hour dust treatment preceded intratracheal administration of fungal spores (treatment groups) or saline solution (tent control group). Goats were exposed to aerosolized dust once weekly for 6 consecutive weeks.

Preparation of fungal spores—Size of the 7 fungal spore types was as follows: *Aspergillus fumigatus* and *Penicillium chrysogenum*, 2 to 5 µm; *Trichoderma viride*, 3 to 4 µm; *Chaetomium globosum* and *Mucor ramosissimus*, 4 to 8 µm; *Stachybotrys chartarum*,[19] 5 to 9 µm; and *Monotospora lanuginosa*,[j] 8 to 16 µm. Six of these fungi are commonly found in feedyard dust,[20] with the exception being *S chartarum*, which is commonly found on grain. All 7 types of fungi used in the study were identified by personnel at a microbiological laboratory[k] that specialized in fungi identification.

Each species of fungus was incubated on 30 malt-extract agar[l] plates (100 × 15 mm) at 28°C for sufficient time (5 to 14 days) to enable development of massive sporulation. Each Petri plate[m] was washed with 5 mL of sterile physiologic saline solution, and contents of the 30 plates were pooled. Sterile saline solution was added to achieve a final volume of 210 mL. To separate spores from hyphae, the fungal suspension was mixed and strained twice through a 30-mm, No. 140 sieve.[n] Concentration of spores in the suspension was counted in a hemacytometer.[o] The spore suspensions were also titrated in triplicate on malt-extract agar Petri plates to calculate the mean number of viable CFUs per milliliter. Antibacterial inhibitors (chlortetracycline,[p] 5 mg/L; streptomycin sulfate,[q] 100 mg/L) were incorporated into malt-extract agar to prevent bacterial growth. Each spore preparation was mixed and divided equally into six 30-mL syringes labeled appropriately for each group of fungi. Weekly for 6 weeks, each spore type was inoculated immediately after goats were exposed to aerosolized dust for 4 hours.

Intratracheal inoculation of fungal spores—Contents of the syringes were mixed thoroughly immediately prior to tracheal inoculation. Initially, goats in 4 treatment groups (*A fumigatus*, *P chrysogenum*, *M ramosissimus*, and *M lanuginosa*) and the tent control group were administered an anesthetic[r] locally at the site of the needle insertion for the intratracheal inoculation, and a sedative (xylazine hydrochloride[s]) was administered IM. Thirty milliliters of the spore preparation (or saline solution for the tent control group) was inoculated into the lumen of the proximal portion of the trachea at a location just distal to the cricoid cartilage. However, several of these goats died as a result of idiopathic reactions related to the sedative.

Therefore, goats in the remaining 3 treatment groups (*C globosum*, *T viride*, and *S chartarum*) were anesthetized for fungal inoculation. Goats were administered atropine sulfate[t] (0.04 mg/kg, IM), which was followed by an IV injection of a combination of butorphanol tartrate[u] (0.5 mg/kg) and ketamine hydrochloride[v] (4.4 mg/kg). A speculum[w] with a light source was inserted into the oral pharynx of each goat, and the epiglottis was viewed. Then a 1-mL pipette[x] equipped with a tip consisting of a hollow rubber tube was inserted past the epiglottis into the proximal portion of the trachea. A syringe containing the fungal spore solution was attached to the pipette, and the 30 mL of spore preparation was inoculated into the trachea.

Table 1—Mean ± SEM number of total, viable, and nonviable fungal spores/30-mL dose administered weekly to each groups of goats (6 goats/group) during a 6-week period.

Fungal type	Viable spores	Nonviable spores	Total spores
Aspergillus fumigatus	$0.495 \times 10^{10} \pm 0.381 \times 10^{10}$	$1.287 \times 10^{10} \pm 0.804 \times 10^{10}$	$1.782 \times 10^{10} \pm 1.052 \times 10^{10}$
Chaetomium globosum	$0.7038 \times 10^{8} \pm 0.2566 \times 10^{8}$	$4.494 \times 10^{8} \pm 1.597 \times 10^{8}$	$5.198 \times 10^{8} \pm 1.509 \times 10^{8}$
Monotospora lanuginosa	$0.030 \times 10^{9} \pm 0.010 \times 10^{9}$	$2.377 \times 10^{9} \pm 1.065 \times 10^{9}$	$2.408 \times 10^{9} \pm 1.066 \times 10^{9}$
Mucor ramosissimus	$0.115 \times 10^{10} \pm 0.689 \times 10^{10}$	$4.649 \times 10^{10} \pm 1.964 \times 10^{10}$	$4.764 \times 10^{10} \pm 1.996 \times 10^{10}$
Penicillium chrysogenum	$0.214 \times 10^{10} \pm 0.026 \times 10^{10}$	$7.068 \times 10^{10} \pm 1.563 \times 10^{10}$	$7.282 \times 10^{10} \pm 1.554 \times 10^{10}$
Stachybotrys chartarum	$0.041 \times 10^{10} \pm 0.02702 \times 10^{10}$	$0.751 \times 10^{10} \pm 0.2644 \times 10^{10}$	$0.7920 \times 10^{10} \pm 0.2894 \times 10^{10}$
Trichoderma viride	$7.831 \times 10^{10} \pm 0.4414 \times 10^{10}$	$5.247 \times 10^{10} \pm 1.418 \times 10^{10}$	$6.030 \times 10^{10} \pm 1.126 \times 10^{10}$

Collection of data and samples—Prior to each inoculation of fungal spores, blood samples were collected from a jugular vein and rectal temperatures[y] were obtained from each goat. Rectal temperatures were obtained 0, 4, 24, 48, and 72 hours after inoculation of fungal spores, and blood samples were collected for WBC counts 24 and 72 hours after inoculation of fungal spores.

Necropsy—At completion of the study, all goats were to be euthanatized by IV administration of an overdose of a barbiturate solution.[z] One goat from each group was euthanatized 24 hours after the sixth inoculation of fungal spores, and the remaining goats in each group were euthanatized 72 hours after the last spore inoculation. All goats were necropsied by a veterinary pathologist (JRA). Gross and histologic examinations of tissues were performed by the pathologist, who was not aware of the group status for each goat.

During necropsy, sterile cotton-tipped applicators[aa] were used to collect mucus and tissue fluid (0.1 mL) from the nasal cavity, thoracic cavity, proximal portion of the trachea, distal portion of the trachea, bronchi, and lungs. Mucus and tissue fluid were expressed from each swab by rotating the swab on the surface of a malt-extract agar plate; mucus and tissue fluid were further dispersed on the surface of the agar by use of the streak dilution technique. The inoculated plates were incubated[bb] at 28°C. A thermophilic fungus (*M lanuginosa*) was isolated by use of similar techniques, but those cultures were incubated[cc] at 50°C. The number of fungal colonies on incubated plates was enumerated 24, 72, and 120 hours after onset of incubation.

Tissue samples from all 6 lung lobes of each goat were collected and immediately placed in neutral-buffered 10% formalin.[dd] The tissues were processed and stained with H&E and by use of the Gomori methenamine silver method.[21]

Statistical analysis—Mean values for measured variables were compared by use of an ANOVA.[22] Mean values for rectal temperature and total WBC counts were compared between treatment and control groups throughout the study and within collection periods for specific sampling days. Significant differences between mean values were also determined by use of the Bonferroni and Dunnett adjusted paired *t* tests, which allowed pairwise comparisons of means for the groups within any day of sample collection. Values for all tests were considered significant at $P \leq 0.05$.

Results

Effects on goats—Seven goats died during the study (2 in the tent control group, 3 in the *M ramosissimus* group, and 2 in the *M lanuginosa* group). One goat in the tent control group died of a perforating ulcer in the urinary bladder, and the other goat in the tent control group died of an idiopathic reaction related to the sedative. Three goats in the *M ramosissimus* group died as a result of an idiopathic drug reaction; 2 of these goats had paralaryngeal cellulitis and abscesses related to deposition of some of the spore inoculations outside the lumen of the trachea (*Mucor* spores were isolated from the abscesses). No goats died during the experimental period after the inoculation technique was changed to tracheal inoculation via a tube inserted through the oral pharynx.

Total and viable spore counts—Mean counts of cultured viable spores were subtracted from the mean counts of total spores to determine the mean number of nonviable spores for each of the 7 fungal species. The mean value for each 30-mL spore suspension of the same spore was calculated for 6 wk/goat, and the mean values for each goat per group were used to calculate the mean ± SEM. Mean total spore count, mean viable spore count, and mean nonviable spore count were determined for each week of the 6-week study for each fungal type of each 30-mL dose/goat (**Table 1**).

Characterization of feedyard dust based on size distribution—Three histograms of the size of particles were extremely similar (data not shown). Particle size ranged from 0.142 to 502 µm (mean, 80 µm).

Response to dust exposure and tracheal spore inoculations—Goats responded calmly to the six 4-hour dust treatments. Goats were covered with dust

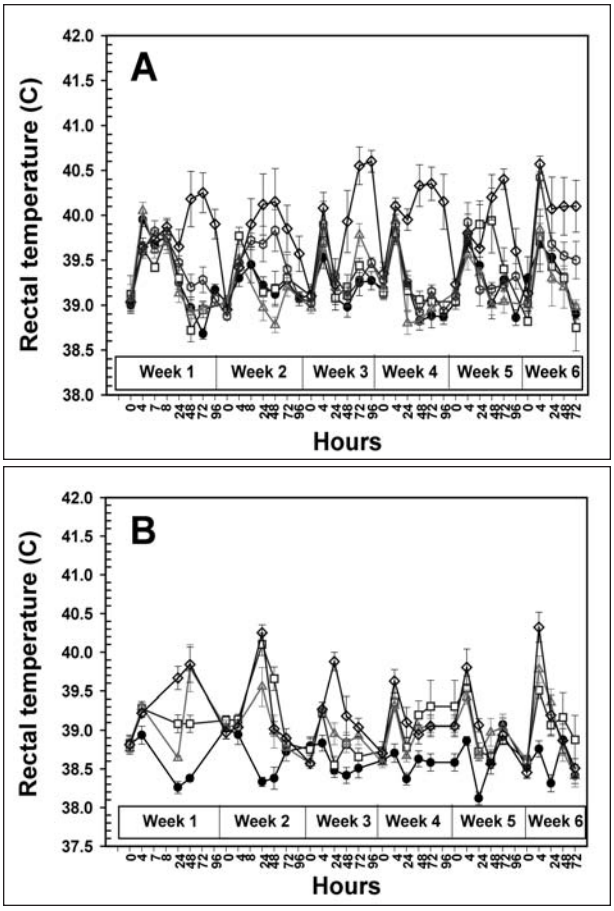

Figure 1—Mean ± SEM rectal temperature for a tent control group (A; solid circles) and each of 4 spore-treated groups (*Aspergillus fumigatus* [open squares], *Monotospora lanuginosa* [open triangles], *Mucor ramosissimus* [open diamonds], and *Penicillium chrysogenum* [open hexagons] and a pen control group (B; solid circles) and each of the 3 remaining spore-treated groups (*Chaetomium globosum* [open squares], *Stachybotrys chartarum* [open triangles], and *Trichoderma viride* [open diamonds]) of goats. Each group initially comprised 6 goats. Goats in each of the treatment groups and the tent control group were exposed to aerosolized sterile feedyard dust for 4 hours prior to intratracheal administration of 30 mL of a fungal spore preparation or 30 mL of physiologic saline (0.9% NaCl) solution (ie, tent control group); dust exposure and intratracheal injections were repeated weekly for 6 weeks. Goats in the pen control group were not exposed to aerosolized sterile dust and did not receive intratracheal injections. Mean rectal temperature of each treatment group was compared with the mean rectal temperature of the respective control group in each panel. Time 0 = Time of intratracheal injection.

when they were removed from the tent after a 4-hour exposure. The internal nares were covered with the dust, which became moistened by vapor from respiration. Some goats had intermittent coughing after they were removed from the tent; however, this coughing subsided within 24 hours. Intratracheal inoculations of the 30-mL suspension of fungal spores were more difficult than had been anticipated. The needle slipped out of the lumen of the trachea in a few instances. *Mucor ramosissimus* spores were prone to induce an abscess when they were deposited outside the lumen of the trachea. Clinically, *M ramosissimus* and *T viride* appeared to be more pathogenic than the other types of fungi. This clinical impression of these 2 fungal types was reinforced by the increase in rectal temperature and by gross and histologic lung lesions.

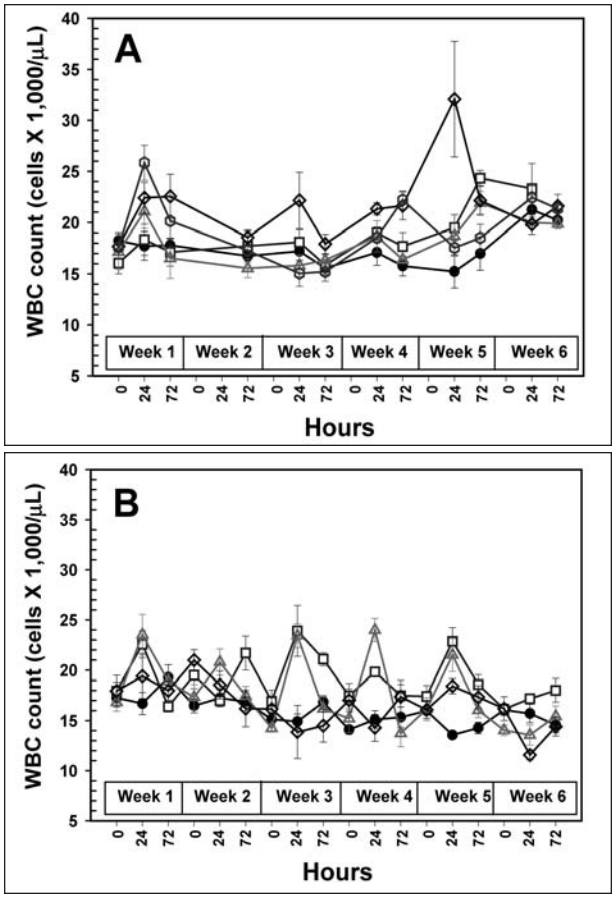

Figure 2—Mean ± SEM total WBC counts for a tent control group (A; solid circles) and each of 4 spore-treated groups (*A fumigatus* [open squares], *M lanuginosa* [open triangles], *M ramosissimus* [open diamonds] and *P chrysogenum* [open hexagons]) and a pen control group (B; solid circles) and each of the 3 remaining spore-treated groups (*C globosum* [open squares], *S chartarum* [open triangles], and *T viride* [open diamonds]) of goats. *See* Figure 1 for remainder of key.

We detected significant ($P < 0.001$) differences in mean rectal temperatures and mean WBC counts among groups following inoculation of the various fungal spores (**Figures 1 and 2**). Mean rectal temperature of each of the spore treatment groups for the first 4 treatment groups (ie, *A fumigatus*, *P chrysogenum*, *M ramosissimus*, and *M lanuginosa*) was compared with mean rectal temperature of the tent control group. For goats inoculated with *M ramosissimus*, mean rectal temperature was significantly increased in weeks 1 through 4 and week 6 at 24, 48, and 72 hours after inoculation. The exception to this pattern was week 5, when the mean rectal temperature decreased at 72 hours. For goats inoculated with *P chrysogenum* spores, mean rectal temperature significantly increased in week 2 (24 and 48 hours after inoculation) and week 6 (72 hours after inoculation). For goats inoculated with *M lanuginosa* spores, mean rectal temperature was significantly increased in week 4 (72 hours after inoculation). Mean rectal temperature for goats inoculated with *A fumigatus* was significantly increased in week 5 at 24 and 48 hours after inoculation.

Mean rectal temperature of the remaining 3 spore treatment groups (ie, *C globosum*, *T viride*, and *S chartarum*) was compared with that of the pen control goats. Goats that received *T viride* spores had a significant increase in mean rectal temperature in week 1 (24 and 48 hours after inoculation), weeks 2 and 3 (24 hours after inoculation), weeks 4 and 5 (4 hours after inoculation), and week 6 (4 and 24 hours after inoculation). For the *C globosum* group, mean rectal temperature was significantly increased in weeks 1 and 2 (24 and 48 hours after inoculation), week 3 (4, 24, and 48 hours after inoculation), week 4 (4, 24, 48, and 72 hours after

Table 2—Mean ± SD size of atelectatic lesions in each group of goats.*

			Right lung			Left lung		
Fungal type	**Severity†**	**No. of goats**	**Cranial**	**Middle**	**Caudal**	**Cranial**	**Middle**	**Caudal**
*M ramosissimus**	1‡	4	4,700 ± 1,536	2,650 ± 1,109	5,745 ± 4,171	1,350 ± 1,559	2,325 ± 862	2,200 ± 2,540
T viride§	2‡	6	3,318 ± 3,859	1,560 ± 1,695	2,964 ± 2,312	7,849 ± 12,593	1,833 ± 1,066	851 ± 696
S chartarum§	3‡	6	2,767 ± 900	1,993 ± 670	3,133 ± 1,876	2,053 ± 1,440	1,656 ± 756	1,201 ± 832
C globosum§	4‡	6	2,781 ± 1,086	2,357 ± 1,418	1,921 ± 1,262	386 ± 665	990 ± 532	190 ± 425
*P chrysogenum**	5	6	2,425 ± 1,611	1,750 ± 920	500 ± 1,225	200 ± 489	333 ± 532	1,650 ± 4,042
*A fumigatus**	6	5	2,142 ± 1,279	1,710 ± 971	150 ± 141	1,350 ± 1,559	2,325 ± 862	2,200 ± 2,540
*M lanuginosa**	7	6	906 ± 1,001	581 ± 1,102	0	0	0	0
Tent control*	8	6	1,133 ± 489	88 ± 161	50 ± 123	33 ± 82	75 ± 184	0
Pen control	9	6	649 ± 1,166	420 ± 698	0	0	97 ± 124	0

Values reported are the number of square millimeters for each lesion, which was obtained by multiplying the width and length of each lesion.
*Each treatment group was exposed to aerosolized sterile dust for 4 hours followed by intratracheal inoculation with 30 mL of a fungal spore suspension weekly for 6 weeks, the tent control group was exposed to aerosolized sterile dust for 4 hours followed by intratracheal injection with 30 mL of physiologic saline (0.9% NaCl) solution weekly for 6 weeks; and the pen control group was not exposed to aerosolized sterile dust and did not receive intratracheal injections. †Severity represents the ranking (greatest severity of lesions to least severity of lesions) and was determined by adding values for lesions in all lung lobes in that group. ‡Values differ significantly ($P \leq 0.05$; Dunnett paired *t* test) from values for the tent control group. §Values for this treatment group were compared with values for the pen control group.

inoculation), week 5 (4, 24, and 48 hours after inoculation), and week 6 (4 and 24 hours after inoculation). Mean rectal temperature was significantly increased for the *S chartarum* group in week 2 (24 and 48 hours after inoculation), week 3 (4, 24, and 72 hours after inoculation), week 4 (4, 24, and 48 hours after inoculation), week 5 (4, 24, and 48 hours after inoculation), and week 6 (4 and 72 hours after inoculation).

Mean WBC count of the initial 4 treatment groups was compared with the mean WBC count of the tent control goats. For goats inoculated with *P chrysogenum*, mean WBC count was significantly increased in week 1 (24 hours after inoculation) and week 4 (72 hours after inoculation). For goats inoculated with *M ramosissimus*, mean WBC count was significantly increased in week 1 (24 and 72 hours after inoculation) and weeks 3 and 5 (24 hours after inoculation). Mean WBC count was significantly increased for goats inoculated with *A fumigatus* in week 5 at 24 and 72 hours after inoculation.

Mean WBC count of the remaining 3 treatment groups was compared with the mean WBC count of the pen control goats. For goats inoculated with *S chartarum*, mean WBC count was increased in weeks 1 through 5 (24 hours after inoculation). For goats inoculated with *C globosum*, the mean WBC count was increased in weeks 1 and 4 (24 hours after inoculation), weeks 2 and 6 (72 hours after inoculation), and weeks 3 and 5 (24 and 72 hours after inoculation). The mean WBC count was increased for goats inoculated with *T viride* in weeks 1 and 5 (24 hours after inoculation) and week 2 (0 hours [time of inoculation]).

The thermophilic fungus *M lanuginosa* appeared to be the least pathogenic of the fungal types. However, 1 goat inoculated with *M lanuginosa* died during the study as a result of bronchopneumonia complicated by rumen acidosis.

Necropsy—Atelectasis was the most common lesion observed during necropsy (**Table 2**). Atelectasis was most prominently observed in goats inoculated with *M ramosissimus*. Consolidated lung lesions were also observed in goats inoculated with *M ramosissimus*, *T viride*, *S chartarum*, and *C globosum*.

Histologic examination of respiratory tract tissues revealed that goats from the tent control and *M lanuginosa* groups had mild, patchy atelectasis with bronchial-associated lymphoid tissue. Fungal spores and small fragments of hyphae were observed in some sections of the goats inoculated with *M lanuginosa*, and no spores or hyphae were observed histologically in lung sections of the tent control group. Tissues from goats inoculated with *M ramosissimus* had an airway exudate consisting of proteinaceous fluid that contained many macrophages and neutrophils, with a few granulomas; some lung sections contained multinucleated giant cells. There was patchy fibrosis in a number of histologic lung sections from goats inoculated with *M ramosissimus*, *A fumigatus*, and *P chrysogenum*. These groups also had patchy to coalescing airway exudate that contained macrophages, neutrophils, and giant cells. There was a noticeable increase in peri–airway-associated lymphoid tissue in these 3 fungal groups, and fungal spores and small fragments of hyphae were observed in some sections. Substantial microscopic lung lesions were observed in the pen control group. Goats inoculated with *C globosum*, *S chartarum*, and *T viride* had patchy to generalized alveolar and airway exudate that contained macrophages and neutrophils, with granulomas and a few giant cells in some sections. Fungal spores and small fragments of hyphae were observed in macrophages in most of the sections examined.

Use of Gomori stain revealed that all lung sections from negative-control goats were negative for fungal elements and that all sections from goats in the treatment groups were positive for fungal elements in mononuclear cells. Fungal spores in lung sections of goats inoculated with *M ramosissimus* or *A fumigatus* had buds and germinal tubules.

Subjectively, severity of lesions differed among the various fungal organisms inoculated into the lungs of goats. In descending order of severity of lesion, the fungal organisms were *M ramosissimus*, *T viride*, *C globosum, S chartarum, A fumigatus, P chrysogenum*, and *M lanuginosa*.

Diagnoses were established for infections resulting from the various fungal organisms. *Mucor ramosissimus* caused chronic exudative mycotic pneumonia with granuloma formation, whereas *A fumigatus* and *P chrysogenum* caused chronic exudative pneumonia. Inoculation of *C globosum*, *S chartarum*, and *T viride* caused chronic active pyogranulomatous pneumonia.

Table 3—Mean ± SD number of fungal isolates cultured from various tissues obtained from goats in each treatment group.

Fungal type	No. of goats		Bronchial lymph	Thoracic fluid	Proximal aspect of trachea	Distal aspect of trachea	Bronchi	Lungs
	24 hours*	72 hours*						
A fumigatus	1†	5	0	0	4.0 ± 8.8	3.0 ± 7.4	0	261 ± 285
C globosum	1	5	0.7 ± (0.5)	0	1.3 ± 2.8	1.7 ± 3	0	71 ± 137
M lanuginosa	2† and 1	4	0	0	95 ± 157	91 ± 159	0	186 ± 422
M ramosissimus	3†	3	0	0	55 ± 68	50 ± 61	0	196 ± 299
P chyrsogenum	1	4	0	0	94 ± 200	18 ± 23	0	51 ± 52
S chartarum	1	5	0.3 ± 0.8	0	4 ± 9	3 ± 7	0	62 ± 95
T viride	1	5	31 ± 23	51 ± 29	0	40 ± 8	67 ± 103	71 ± 27
Tent control	2†	4	0	0	0	0	0	0
Pen control	1	5	0	0	0	0	0	0

Values reported represent number of CFUs/0.1 mL of sample.
*Samples were obtained 24 or 72 hours after the last of 6 weekly 4-hour exposures to aerosolized sterile feedyard dust followed by intratracheal inoculation with a fungal spore suspension (7 treatment groups) or intratracheal injection with 30 mL of physiologic saline solution (tent control group); the pen control group was not exposed to aerosolized sterile dust and did not receive intratracheal injections. †These goats died; all other goats were euthanatized.

Isolation of fungi from respiratory tracts—Various amounts of viable spores were frequently recovered from the respiratory tract of goats of all 7 treatment groups (**Table 3**). Isolation of fungi from the nasal cavity was not reported because it frequently contains environmental fungi of all types. The bronchial lymph nodes and thoracic fluid of all goats inoculated with *T viride* yielded fungal spores.

Discussion

Environmental fungi can induce disease in domestic animals and humans via infections on[23] or in a host,[24-26] by inducing allergic reactions initiated by inhalation of dead or living fungus spores or fragments of hyphae,[27-30] and through production of toxins.[31-34] Toxins are most often introduced into a host through ingestion of contaminated foods.[35] In humans, it has been proposed[36] that toxic effects can be induced by inhalation of fungal toxins; however, this hypothesis is controversial.

When small dust particles of low toxicity fill alveolar macrophages deep in the lungs, the phagocytes may be overloaded and impaired. One hypothesis is that overloading impairment is caused by suppressed pulmonary clearance and there is a concurrent recruitment of alveolar macrophages and neutrophils in the alveolar area.[37] In addition to overloading, macrophages may also be damaged by toxins, mycotoxins, and proteolytic enzymes from the phagocytized dust and microbes.[14] There is an increase in the number of particles that enter the interstitial space.[38] The percentage volume of particles may be more important than the number of particles in overloading of alveolar macrophages.[39] Pulmonary clearance of ultrafine particles is much slower than for larger particles.[40] There may be a prolonged inflammatory response with persistent and excessive elaboration of chemotatic substances and an elaboration of proinflammatory cytokines.[41] This may allow fungal spores to escape phagocytosis. It is also conceivable that fungal spores could infect or release immunosuppressive mycotoxins[16,17] that may damage the delicate lung tissues. The possible overloading of alveolar macrophages with sterilized feedyard dust may decrease the immune response sufficiently to allow inoculated fungal spores to induce tissue damage prior to their elimination from the lungs. The endotoxin in organic manure dust activates macrophages through CD14 receptor sites.[42] Host pulmonary defenses to *Rhizopus* and *Aspergillus* vary even in clinically normal animals. Host defenses against *Aspergillus* spores depend on early killing of the spores by alveolar macrophages, whereas the prevention of mucormycosis appears to require that bronchioalveolar macrophages inhibit the conidia from converting into the hyphal form; however, these macrophages are unable to kill *Rhizopus oryzae* spores.[43]

The study reported here revealed which of the 7 types of spores was the most toxic when infused 6 times into the trachea of nonimmunosuppressed goats. Histologic examination of lung tissues collected from each of the 7 groups revealed that the fungal elements (spores and small fragments of hyphae) were evident 24 and 72 hours after inoculation of spores into the trachea. It has been reported[44] that viable *P chrysogenum* spores were not cultured from the lungs of mice when samples were obtained > 36 hours after exposure. Spore buds and germination tubules were seen twice in 1 goat from each of the *A fumigatus* and *M ramosissimus* groups.

Size of the fungal spores ranged from 2 to 7 µm for 6 of the 7 fungal groups and was 8 to 16 µm for *M lanuginosa*. It would appear that the upper end of the range of the spore sizes could not possibly reach the alveoli in humans; however, it appears that some spores of all 7 groups reached the alveoli in goats. *Montospora lanuginosa* had the largest spore size and also had the least severity of observed lung lesions (not significantly different from the severity for the control groups). Nevertheless, *M lanuginosa* spores were observed in the alveolar region. Perhaps there is a wider range of spore sizes for this fungi, especially smaller spores, than has been published. Certainly, viable spores were isolated from the lung tissues of all 7 groups of goats.

Of the 7 groups of fungi tested, the most invasive appeared to be *T viride* as determined on the basis of its isolation in greatest numbers from the thoracic fluid and thoracic lymph nodes of all goats in that group. *Trichoderma* organisms were isolated from both lungs in 5 of 6 goats, the exception being that organisms were not isolated from the middle and caudal lobes of the left lung of 1 goat. A few fungal CFUs were isolated from the bronchial lymph nodes of at least 1 goat that was inoculated with *C globosum* and *S chartarum*, and no fungi were isolated from the thoracic fluid of any of the 7 groups. *Mucor ramosissimus* was extremely pathogenic when inadvertently deposited into the soft tissues of the neck, and it caused large abscesses that probably contributed to the deaths of 2 goats. *Mucor* organisms inoculated via the trachea also induced severe damage to the ventral half of all 3 lobes of the right lung in 4 goats and to all 3 lobes of the left lung in 2 of those goats.

The mycotoxin for *Penicillium* and *Aspergillus* (excluding aflatoxins that induce aflatoxicosis in birds and farm animals that eat contaminated food) appears to be of low toxicity.[45] Gliotoxin and helvolic acid reportedly[46] are produced by *A fumigatus*. *Penicillium verrucosum* and *Aspergillus ochraceus* produce the mycotoxin ochratoxin A.[47] We were not able to find any information on mycotoxin production by *Monotospora* organisms.

Four treatment groups (*M ramosissimus*, *T viride*, *S chartarum*, and *C globosum*) had significantly larger atelectatic and consolidated lung lesions, compared with lesions for the other 3 treatment groups or the 2 control groups. Excluding *M ramosissimus*, 3 of these fungal groups produce mycotoxins.[31,48-50] *Stachybotrys chartarum*, an important fungus with a worldwide distribution, produces macrocyclic trichothecenes referred to as satratoxins G and H[51] that are potent inhibitors of protein and DNA synthesis.[52] It grows well in plaster wallboards,[53] ceiling tiles, and particle boards from houses that have been damaged by water leaking through roofs or water pipes. It is frequently called black mold by the insurance industry, and most, if not all, insurance companies no longer cover this mold infestation in their common homeowner policies because of the number of claims that were made. People who remain in houses with this mold are at risk of serious health deterioration, probably through inhalation[51] of fungal elements that

contain the mycotoxin. This fungus is believed, but not proven, to induce infant idiopathic pulmonary hemorrhage syndrome.[54,55] *Trichoderma* produces trichothecene mycotoxins,[56] which are frequently referred to as trichodermol and its acetyl ester trichodermin. *Chaetomium globosum* produces the highly toxic chaetomins and chaetoglobosins mycotoxins.[57]

The exception appears to be *M ramosissimus*. We were not able to find anything on mycotoxin production by this organism in the literature. One publication[58] on *Zygomycetes* in humans indicated that there are 2 orders of *Zygomycetes* (*Mucorales* and *Entomophthorales*) and that most of the illnesses were caused by *Mucorales*. *Mucorales* includes *Rhizopus* spp. Other fungi associated with human illness were *Mucor, Rhizomucor, Absidia*, and 5 other fungal types. Infections with *Mucorales* organisms commonly lead to thrombosis and infarctions of involved tissues and result in tissue destruction that is often mediated by a number of fungal proteases, lipases, and mycotoxins. The spores from these fungi are transmitted by inhalation or ingestion.[58] Infection with *Mucor* spp results in an extremely aggressive mucormycosis in immunocompromised humans[59] and can also induce infections in humans that are not immunocompromised.[60] Mucormycosis has been diagnosed in many animal species, including marine mammals,[61] cattle,[62] sheep,[63] swine,[64] horses,[65] platypuses,[66] tree frogs,[67] and birds.[68]

Spores from 4 types of fungi (*M ramosissimus*, *T viride*, *C globosum*, and *S chartarum*) induced substantial pathologic changes in the lungs when healthy goats received repeated intratracheal inoculations. It is suspected that the pathologic changes, atelectasis, and consolidated lesions were caused by fungal exoproducts (ie, proteases, lipases, and mycotoxins). These substances were probably released when spores were destroyed by the host macrophages and neutrophils; alternatively, they could have been actively produced by the spores. Additional investigations will be necessary to provide answers. The atelectatic lung lesions observed in the tent control goats were induced by exposure to aerosolized sterile dust prior to intratracheal injection of 30 mL of sterilized physiologic saline solution. Similar lesions were not seen in the pen control goats that were not exposed to aerosolized dust or administered saline solution via intratracheal injections.

a. Ivomec, MSD AGVET, Merck & Co Inc, Rahway, NJ.
b. Amprolium, MSD AGVET, Merck & Co Inc, Rahway, NJ.
c. Malvern Mastersizer 2000, version 5.1, Malvern Instruments Ltd, Malvern, UK.
d. Autoclave, Amsco 2021 eagle series, Steris Corp, Healthcare Division, Erie, Pa.
e. Custom-fabricated canvas tent, Wolfe Canvas, Amarillo, Tex.
f. AccuRate dry material feeder, Hopper, Whitewater, Wis.
g. Jet-O-Mizer, Fluid Energy Processing & Equipment Co, Hatfield, Pa.
h. Gasoline-powered portable air compressor, Stewart-Warner, Johnson City, Tenn.
i. Portable blower and vacuum motor (3 horse power), Grainger, Amarillo, Tex.
j. Crisan V. *The isolation and identification of thermophilic fungi*. Master of Science thesis, Department of Microbiology, Purdue University, West LaFayette, Ind, 1959;60–73.
k. Center for Indoor Air Research, Health Sciences Center, Texas Tech University, Lubbock, Tex.
l. Malt-extract agar, Difco Laboratories, Detroit, Mich.
m. Falcon plastic Petri plates, Becton, Dickinson & Co, Franklin Lakes, NJ.
n. Standard test sieve, No. 140 (ASTM specifications), EH Sargent & Co, Chicago, Ill.
o. Bright line hemacytometer, Hausser Scientific, Horsham, Pa.
p. Chlortetracycline, Sigma Chemical Co, St Louis, Mo.
q. Streptomycin sulfate, Sigma Chemical Co, St Louis, Mo.
r. Lidocaine HCL, 2% solution, RX Veterinary Products, Grapevine, Tex.
s. Xylazine HCL injection, 100 mg/mL, 50-mL vial, RX Veterinary Mfg by Phoenix, St Joseph, Mo.
t. Atropine sulfate LA, 15 mg/mL, 100-mL vial, Neogen Corp, Lexington, Ky.
u. Butorphanol tartrate, 10 mg/mL, 50-mL vial, Fort Dodge Animal Health, Fort Dodge, Iowa.
v. Ketamine HCL, 100 mg/mL, 10-mL vial, Fort Dodge Animal Health, Fort Dodge, Iowa.
w. Custom-fabricated speculum made of 2.7 × 20-cm polyvinyl chloride pipe fitted with a clip pen light, USDA-Agricultural Research Service, Conservation and Production Research Laboratory, Bushland, Tex.
x. Falcon plastic 0.1-mL pipettes, Becton, Dickinson & Co, Franklin Lakes, NJ.
y. Rectal thermometer, hi-speed digital rechargeable, GLA, San Luis Obispo, Calif.
z. Beuthanasia-D Special, Schering-Plough Animal Health, Union, NJ.
aa. Cotton-tipped plastic applicators, Puritan 25-806-2PC, Hardwood Products Co, Guilford, Mass.
bb. Custom-fabricated walk-in environmental incubator, USDA-Agricultural Research Service, Conservation and Production Research Laboratory, Bushland, Tex.
cc. Carbon dioxide water-jacketed incubator, Forma Scientific, Marietta, Ohio.
dd. Formaldehyde, 37 wt% solution in water, ACS Reagent, Aldrich, Milwaukee, Wis.

References

1. Purdy CW, Straus DC, Chirase N, et al. Effects of aerosolized feedyard dust that contains natural endotoxins on adult sheep. *Am J Vet Res* 2002;63:28–35.
2. Purdy CW, Straus DC, Parker DB, et al. Treatment of feedyard dust containing endotoxin and its effect on weanling goats. *Small Rumin Res* 2002;46:123–132.
3. Cormack DH. The respiratory system. In: Cormack DH, ed. *Ham's histology*. 9th ed. Philadelphia: JB Lippincott Co, 1987;541–566.
4. Singh PK, Tack BF, McCray PB Jr, et al. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L799–L805.
5. Boyton RJ, Openshaw PJ. Pulmonary defences to acute respiratory infection. *Br Med Bull* 2002;61:1–12.
6. Zhang P, Summer WR, Bagby GJ, et al. Innate immunity and pulmonary host defense. *Immunol Rev* 2000;173:39–51.
7. Andersen AA. New sampler for the collection, sizing and enumeration of viable airborne particles. *J Bacteriol* 1958;76:471–484.
8. Oberdorster G, Ferin J, Morrow PE. Volumetric loading of alveolar macrophages (AM): a possible basis for diminished AM-mediated particle clearance. *Exp Lung Res* 1992;18:87–104.
9. Negishi T. Lung clearance of particles following excessive deposition of fly ash in golden hamsters. *Exp Anim* 1995;44:131–138.
10. Vincent JH, Donaldson K. A dosimetric approach for relating the biological response of the lung to the accumulation of inhaled mineral dust. *Br J Ind Med* 1990;47:302–307.
11. Moore BB, Moore TA, Toews GB. Role of T- and B-lymphocytes in pulmonary host defences. *Eur Respir J* 2001;18:846–856.
12. Mansour MK, Levitz SM. Interactions of fungi with phagocytes. *Curr Opin Microbiol* 2002;5:359–365.
13. Oberdorster G. Lung particle overload: implications for occupational exposures to particles. *Regul Toxicol Pharmacol* 1995; 21:123–135.
14. Morrow PE. Dust overloading of the lungs: update and appraisal. *Toxicol Appl Pharmacol* 1992;113:1–12.
15. Morrow PE. Possible mechanisms to explain dust overloading of lungs. *Fundam Appl Toxicol* 1988;10:369–384.

16. Watanabe A, Kamei K, Sekine T, et al. Immunosuppressive substances in *Aspergillus fumigatus* culture filtrate. *J Infect Chemother* 2003;9:114–121.

17. Reijula K, Tuomi T. Mycotoxins of aspergilli: exposure and health effects. *Front Biosci* 2003;8:s232–s235.

18. Purdy CW, Straus DC, Chirase N, et al. Effect of aerosolized endotoxin in feedyard dust on weanling goats. *Small Rumin Res* 2002;46:133–187.

19. Larone DH. Part II: detailed description. In: Larone DH, ed. *Medically important fungi: a guide to identification*. 4th ed. Washington, DC: ASM Press, 2002;53–185.

20. Purdy CW, Straus DC, Parker DB, et al. Comparison of the type and number of microorganisms and concentration of endotoxin in the air of feedyards in the Southern High Plains. *Am J Vet Res* 2004;65:45–52.

21. Sheehan DC, Hrapchak BB. Microoganisms. In: Sheehan DC, Hrapchak BB, eds. *Theory and practice of histotechnology*. 2nd ed. St Louis: CV Mosby Co, 1980;233–251.

22. *SAS user's guide: statistics, version 6.03 edition*. Cary, NC: SAS Institute Inc, 1988.

23. Hainer BL. Dermatophyte infections. *Am Fam Physician* 2003;67:101–108.

24. Mandal PC, Gupta PP. Sequential pathological studies in goats infected intratracheally with *Aspergillus fumigatus*. *Mycopathologia* 1993;121:77–81.

25. Mandal PC, Gupta PP. Experimental aspergillosis in goats: clinical, haematological and mycological studies. *Zentralbl Veterinarmed [B]* 1993;40:283–286.

26. Sutton P, Waring P, Mullbacher A. Exacerbation of invasive aspergillosis by the immunosuppressive fungal metabolite, gliotoxin. *Immunol Cell Biol* 1996;74:318–322.

27. Horner WE, Helbling A, Salvaggio JE, et al. Fungal allergens. *Clin Microbiol Rev* 1995;8:161–179.

28. Hebling A. Animals and fungi as allergy inducers. *Ther Umsch* 2001;58:297–303.

29. Boulet LP, Turcotte H, Laprise C, et al. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy* 1997;27:52–59.

30. Nolles G, Hoekstra MO, Schouten JP, et al. Prevalence of immunoglobulin E for fungi in atopic children. *Clin Exp Allergy* 2001;31:1564–1570.

31. Bennett JW, Klich M. Mycotoxins. *Clin Microbiol Rev* 2003;16:497–516.

32. Karlson-Stiber C, Persson H. Cytotoxic fungi—an overview. *Toxicon* 2003;42:339–349.

33. Khan NN, Wilson BL. An environmental assessment of mold concentrations and potential mycotoxin exposures in the greater Southeast Texas area. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2003;38:2759–2772.

34. Creppy EE. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol Lett* 2002;127:19–28.

35. Cleveland TE, Dowd PF, Desjardins AE, et al. United States Department of Agriculture-Agricultural Research Service research of pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Manag Sci* 2003;59:629–642.

36. Fung F, Huyghson WG. Health effects of indoor fungal bioaerosol exposure. *Appl Occup Environ Hyg* 2003;18:535–544.

37. Tran CL, Buchanan D, Cullen RT, et al. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. *Inhal Toxicol* 2000;12:1113–1126.

38. Yu CP, Chen YK, Morrow PE. An analysis of alveolar macrophage mobility kinetics at dust overloading of the lungs. *Fundam Appl Toxicol* 1989;13:452–459.

39. Donaldson K, Bolton RE, Jones A, et al. Kinetics of the bronchoalveolar leucocyte response in rats during exposure to equal airborne mass concentrations of quartz, chrysotile asbestos, or titanium dioxide. *Thorax* 1988;43:525–533.

40. Ferin J, Oberdorster G, Penney DP. Pulmonary retention of ultrafine and fine particles in rats. *Am J Respir Cell Mol Biol* 1992;6:535–542.

41. Monn C, Naef R, Koller T. Reactions of macrophages exposed to particles < 10 μm. *Environ Res* 2003;91:35–44.

42. Tobias PS, Ulevitch RJ. Lipopolysaccharide binding protein and CD14 in LPS-dependent macrophage activation. *Immunobiology* 1993;187:227–232.

43. Waldorf AR. Pulmonary defense mechanisms against opportunistic fungal pathogens. *Immunol Ser* 1989;47:243–271.

44. Cooley JD, Wong WC, Jumper CA, et al. Cellular and humoral responses in an animal model inhaling *Penicillium chrysogenum* spores, in *Proceedings*. 3rd Int Conf Bioaerosols Fungi Mycotoxins 1999;403–410.

45. Nielsen KF. Mycotoxin production by indoor molds. *Fungal Genet Biol* 2003;39:103–117.

46. Fox M, Gray G, Kavanagh K, et al. Detection of *Aspergillus fumigatus* mycotoxins: immunogen synthesis and immunoassay development. *J Microbiol Methods* 2004;56:221–230.

47. Skaug MA, Eduard W, Stromer FC. Ochratoxin A in airborne dust and fungal conidia. *Mycopathologica* 2000;151:93–98.

48. Bhatnagar D, Yu J, Ehrlich KC. Toxins of filamentous fungi. *Chem Immunol* 2002;81:167–206.

49. Abdel-Hafez SI, Shoreit AA. Mycotoxins producing fungi and mycoflora of air-dust from Taif, Saudi Arabia. *Mycopathologia* 1985;92:65–71.

50. Abdel-Hafez SI, Shoreit AA, Abdel-Hafez AL, et al. Mycoflora and mycotoxin-producing fungi of air-dust particles from Egypt. *Mycopathologia* 1986;93:25–32.

51. Sorenson WG, Frazer DG, Jarvis BB, et al. Trichothecene mycotoxin in aerosolized conidia of *Stachybotrys atra*. *Appl Environ Microbiol* 1987;53:1370–1375.

52. Chapman JA. *Stachybotrys chartarum* (chartarum=atra=alternans) and other problems caused by allergenic fungi. *Allergy Asthma Proc* 2003;24:1–7.

53. Murtoniemi T, Nevalainen A, Suutari M, et al. Effect of liner and core materials of plasterboard on microbial growth, spore-induced inflammatory responses, and cytotoxicity in macrophages. *Inhal Toxicol* 2002;14:1097–1101.

54. Hossain MA, Ahmed MS, Ghannoum MA. Attributes of *Stachybotrys chartarum* and its association with human disease. *J Allergy Clin Immunol* 2004;113:200–208.

55. Dearborn DG, Yike I, Sorenson WG, et al. Overview of investigations into pulmonary hemorrhage among infants in Cleveland, Ohio. *Environ Health Perspect* 1999;107(suppl 3):495–499.

56. Godtfredsen WO, Vangedal S. Trichodermin, a new antibiotic, related to trichothecin. *Proc Chem Soc (Lond)* 1964;Jun:161–200.

57. Udagawa S, Muroi T, Kurata H, et al. The production of chaetoglobosins, sterigmatocystin, O-methylsterigmatocystin, and chaetocin by *Chatomium spp* and related fungi. *Can J Microbiol* 1979;25:170–177.

58. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. *Clin Microbiol Rev* 2000;13:236–301.

59. Eucker J, Sezer O, Graf B, et al. Mucormycoses. *Mycoses* 2001;44:253–260.

60. Fairley C, Sullivan TJ, Bartley P, et al. Survival after rhino-orbital-cerebral mucormycosis in an immunocompetent patient. *Ophthalmology* 2000;107:555–558.

61. Sweeney JC, Migaki G, Vainik PM, et al. Systemic mycoses in marine mammals. *J Am Vet Med Assoc* 1976;169:946–948.

62. Jensen HE, Aalbaek B, Schonheyder H. Immunohistochemical identification of aetiological agents of systemic bovine zygomycosis. *J Comp Pathol* 1994;110:65–77.

63. Carrigan MJ, Small AC, Perry GH. Ovine nasal zygomycosis caused by *Conidiobolus incongruous*. *Aust Vet J* 1993;70:239.

64. Sanford SE. Gastric zygomycosis (mucormycosis) in 4 suckling pigs. *J Am Vet Med Assoc* 1985;186:393–394.

65. Guillot J, Collobert C, Jensen HE, et al. Two cases of equine mucormycosis caused by *Absidia corymbifera*. *Equine Vet J* 2000;32:453–456.

66. Whittington RJ, Connolly JH, Obendorf DL, et al. Serological responses against the pathogenic dimorphic fungus *Mucor amphibiorum* in populations of platypus (*Ornithorhynchus anatinus*) with and without ulcerative mycotic dermatitis. *Vet Microbiol* 2002;87:59–71.

67. Creeper JH, Main DC, Berger L, et al. An outbreak of mucormycosis in slender tree frogs (*Litoria adelensis*) and white-lipped tree frogs (*Litoria infrafrenata*). *Aust Vet J* 1998;76:761–762.

68. Dawson CO, Wheeldon EB, McNeil PE. Air sac and renal mucormycosis in an African gray parrot (*Psittacus erithacus*). *Avian Dis* 1976;20:593–600.