

AN EPIDEMIC OF *MALASSEZIA PACHYDERMATIS* IN AN INTENSIVE CARE NURSERY ASSOCIATED WITH COLONIZATION OF HEALTH CARE WORKERS' PET DOGS

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**ABSTRACT**

**Background** *Malassezia* species are lipophilic yeasts that are emerging as nosocomial pathogens, particularly in low-birth-weight neonates who receive lipid emulsions. When a cluster of patients with *Malassezia pachydermatis* infection was identified in an intensive care nursery, we initiated an investigation.

**Methods** A case patient was defined as any infant in the intensive care nursery who had a positive culture for *M. pachydermatis* between October 17, 1993, and January 18, 1995. We conducted a cohort study to identify risk factors for colonization and infection with *M. pachydermatis*. We collected cultures from the infants and the health care workers and from the health care workers' pets, since this organism has been associated with otitis externa in dogs.

**Results** Fifteen infants met the case definition: eight with bloodstream infections, two with urinary tract infections, one with meningitis, and four with asymptomatic colonization. The case patients were significantly more likely than the other infants to weigh 1300 g or less (15 of 65 vs. 0 of 419,  $P < 0.001$ ). In a multivariate analysis of infants weighing 1300 g or less, the independent risk factors for colonization or infection with *M. pachydermatis* were a greater severity of concomitant illness (odds ratio, 19.7;  $P = 0.001$ ), arterial catheterization for nine or more days (odds ratio, 29.5;  $P = 0.02$ ), and exposure to Nurse A (odds ratio, 74.7;  $P = 0.01$ ). In a point-prevalence survey, 9 additional infants, 1 health care worker, and 12 of the health care workers' pet dogs had positive cultures for *M. pachydermatis*. The isolates from all 15 case patients, the 9 additional colonized infants, 1 health care worker, and 3 of the 12 dogs had identical patterns of restriction-fragment-length polymorphisms.

**Conclusions** In this outbreak, it is likely that *M. pachydermatis* was introduced into the intensive care nursery on health care workers' hands after being colonized from pet dogs at home. The organism persisted in the nursery through patient-to-patient transmission. (N Engl J Med 1998;338:706-11.)

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**D**URING the past decade, *malassezia* species have emerged as increasingly important pathogens in neonates in intensive care nurseries. These organisms are lipophilic and have been associated with bloodstream infections in low-birth-weight neonates receiving lipid emulsions. There have been numerous outbreaks caused by *Malassezia furfur* (which is obligately lipophilic), and one outbreak involving *M. pachydermatis* (which is not obligately lipophilic) has been reported.<sup>1</sup> In that outbreak, patient-to-patient transmission of *M. pachydermatis* was documented in an intensive care nursery, but the source of the outbreak was not identified. *M. pachydermatis* has been associated with otitis externa in dogs.

In November 1993, *M. pachydermatis* was cultured from the tip of an intravascular catheter that had been used in an infant in the intensive care nursery at the Dartmouth-Hitchcock Medical Center in Lebanon, New Hampshire. In November and December 1993, two patients in the intensive care nursery had *M. pachydermatis* bloodstream infections. In 1994, colonization or infection with *M. pachydermatis* was documented in 11 more infants. After repeated point-prevalence surveys had identified only one additional infant and no health care workers with *M. pachydermatis* colonization, an investigation was begun.

**METHODS**

**Definition and Ascertainment of Cases**

A case patient was defined as any patient in the intensive care nursery who had a positive culture for *M. pachydermatis* during the study period, which was the period from October 17, 1993 (the date of hospital admission of the first case patient), to Janu-

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ary 18, 1995 (the date of the positive culture from the last case patient). To identify case patients, we reviewed admission and medical records for patients in the intensive care nursery and records of the microbiology department for the study period.

### Epidemiologic Studies

To determine the extent of colonization and infection with *M. pachydermatis*, we conducted an initial cohort study comparing case patients with all other patients who were admitted to the intensive care nursery during the study period. To identify risk factors for colonization and infection with *M. pachydermatis* in infants weighing 1300 g or less, we conducted a second, more detailed cohort study of all infants weighing 1300 g or less who were hospitalized in the intensive care nursery for at least two weeks during the study period (all case patients had been admitted to the nursery at least two weeks before *M. pachydermatis* colonization). Risk factors evaluated included demographic characteristics, birth history, Apgar scores, underlying disease, severity of illness at admission as measured by the Score for Neonatal Acute Physiology,<sup>2,3</sup> clinical signs and symptoms of infection, type and duration of intravascular catheterization, exposure to parenteral and enteral feedings or antimicrobial agents, bed location, and exposure to specific health care workers.

To identify risk factors for colonization and infection with *M. pachydermatis* in health care workers, we obtained culture specimens from the hands of health care workers who had worked in the intensive care nursery during the study period and compared various characteristics of the workers according to whether the cultures were positive or negative. The characteristics included duties; history of skin disease or exposure to antimicrobial agents or glucocorticoids; pet ownership, type of pet (or pets), and illness in a pet (or pets); and hand-washing practices in the intensive care nursery.

### Procedural Investigation

We examined the patient-care areas and other areas in the intensive care nursery, reviewed infection-control and isolation policies and practices, surveyed health care workers about hand-washing practices, and conducted two surreptitious observational studies of health care workers (one before and one after the hand-washing survey), in which we noted the number of contacts with patients and hand-washing practices before and after each contact. In addition, we observed practices associated with total parenteral nutrition and lipid administration, placement of peripheral intravascular catheters, and venipuncture.

### Laboratory Studies

Although most cultures were processed at the Dartmouth-Hitchcock Medical Center laboratory, fungal cultures were sent to a laboratory at a neighboring hospital. Laboratory records were reviewed at both hospitals for all *M. pachydermatis*-positive cultures during the study period. All available *M. pachydermatis* isolates were obtained and sent to the Centers for Disease Control and Prevention (CDC).

### Culture Surveys and Microbiologic Methods

On February 27, 1995, we used sterile swabs premoistened with sterile saline to obtain culture specimens from the face, neck, groin, back, anus, and intravascular-catheterization sites in all 23 infants in the intensive care nursery at the time. We also obtained culture specimens from the hands of all nine health care workers present in the intensive care nursery at the change of shifts, using premoistened sterile towelettes as previously described.<sup>4</sup> On March 1, 3, and 4, 1995, we obtained culture specimens from the ears of 53 pets of health care workers (i.e., dogs, cats, and horses) using premoistened swabs; skin scrapings were obtained from selected dogs.

All specimens were shipped to the CDC, where they were inoculated onto Dixon's malassezia medium, which had been prepared

at the CDC; incubated at 35°C for 48 hours; and then incubated for an additional six days at room temperature. Visible colonies were selected, and wet mounts prepared for microscopical examination. Yeast cells exhibiting a bottle shape, unipolar budding, and collarettes at the apexes of the phialides were tentatively identified as malassezia species. These specimens were subcultured on Dixon's malassezia medium and Sabouraud's dextrose agar (BBL Microbiology Systems, Cockeysville, Md.). Isolates that grew on both mediums were identified as *M. pachydermatis*. All *M. pachydermatis* isolates were sent in blinded duplicates to St. John's Institute of Dermatology, London, where protoplasts were prepared according to the method of Varma and Kwon-Chung,<sup>5</sup> and the nucleic acids extracted according to the method of Scherer and Stevens.<sup>6</sup>

### Statistical Analysis

Data were collected on standardized forms and analyzed with the use of the Epi Info program, version 6.02.<sup>7</sup> Categorical variables were compared with the use of Fisher's exact test or the chi-square test. Continuous variables were compared with Student's t-test. P values of less than 0.05 were considered to indicate statistical significance. To identify independent risk factors, significant factors in the univariate analysis were entered into a multivariate analysis with the use of the PC-SAS program, 3rd edition.<sup>8</sup>

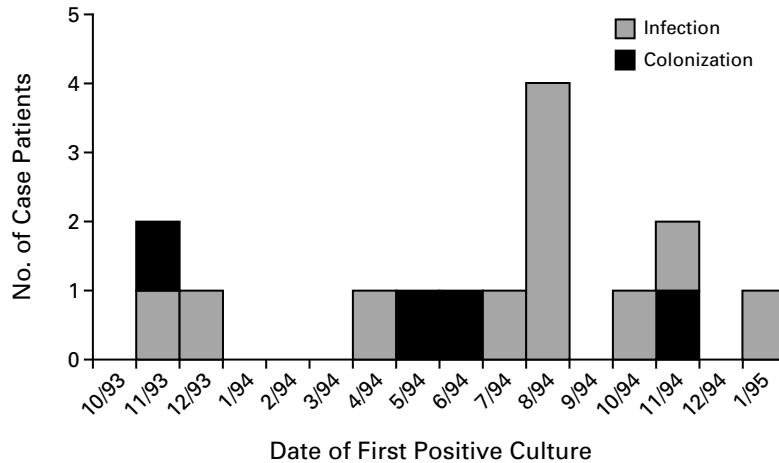
## RESULTS

### Description of Case Patients

Fifteen infants met the definition of case patients (Fig. 1). Eight case patients had bloodstream infections, two had urinary tract infections (positive urine cultures obtained by straight catheterization, without examination of sediment), and one had meningitis (a positive cerebrospinal fluid culture and a negative Gram's stain, with a white-cell count of 89 per cubic millimeter, a glucose level of 2.3 mmol per liter [42 mg per deciliter], and a protein level of 841 mg per deciliter). The other four patients had *M. pachydermatis* colonization of tracheal aspirate (in two patients), skin (in one), and the tip of the intravascular catheter (in one).

The patients ranged in age from 6 to 36 days (median, 21); their gestational age ranged from 24 to 31 weeks (median, 26). All but one of the patients were white; nine were male. Four infants were born from twin gestations, and 10 were born by cesarean section. Thirteen infants had signs or symptoms of infection, the most common being fever (in 11 infants), color change (in 5), the need for intubation or reintubation (in 5), and tachycardia (in 5).

All eight patients with fungemia received intravenous amphotericin B therapy for at least 10 days. In seven of the eight (88 percent), the intravascular catheter was removed or changed. One of the two infants with urinary tract infection and one of the four with colonization but no signs of infection also received amphotericin B. Neither total parenteral nutrition nor administration of lipid emulsion was discontinued in any of the infants. One infant subsequently died from causes unrelated to the infection (respiratory distress syndrome, sepsis, and bowel perforation).



**Figure 1.** Distribution of Case Patients According to the Date of the First Positive Culture for *M. Pachydermatitis*, during the Period from October 1993 through January 1995.

**Assessment of Risk Factors**

In the initial cohort study of 484 infants, the case patients were significantly more likely than the other infants to have a birth weight of 1300 g or less (15 of 65 vs. 0 of 419,  $P < 0.001$ ).

Since all the case patients weighed 1300 g or less, we next focused on the cohort of infants weighing 1300 g or less. Case patients were similar to non-case patients in gestational age, sex, type of delivery, and presence and duration of umbilical or peripheral intravascular catheterization. The case patients had longer stays in the intensive care nursery than did the non-case patients (median stay, 94.5 days vs. 67 days), although this difference was not statistically significant. As compared with the non-case patients, the case patients had a lower median Apgar score at one minute, a higher median Score for Neonatal Acute Physiology, and longer periods of treatment with total parenteral nutrition, lipid emulsion, and antimicrobial agents; they were also more likely to have had central venous or arterial catheterization for a longer period and were more likely to have been exposed to Nurse A or Nurse B (Table 1). When we assessed the independent importance of each of these risk factors by multivariate analysis, only a higher Score for Neonatal Acute Physiology, arterial catheterization for more than nine days, and exposure to Nurse A remained significant (Table 2).

**Hand-Washing Practices and Culture Surveys**

Fifty-four nurses, 4 nurse practitioners, 15 house officers, 2 attending physicians, 4 technicians, 1 secretary, and 1 social worker completed hand-washing questionnaires. Two respondents reported psoriasis, 3 eczema, 2 seborrhea, and 26 other skin disorders. Hand washing before and after contact with patients

**TABLE 1.** RISK FACTORS FOR COLONIZATION AND INFECTION WITH *M. PACHYDERMATIS* AMONG PATIENTS IN AN INTENSIVE CARE NURSERY WHO WEIGHED 1300 g OR LESS AT BIRTH, OCTOBER 1993 THROUGH JANUARY 1995.

RISK FACTOR	CASE PATIENTS (N=15)	NON-CASE PATIENTS (N=50)	RELATIVE RISK (95% CI)*	P VALUE
Central venous catheterization for >9 days (no. of patients)	12	16	5.3 (1.7–17.0)	0.001
Arterial catheterization for >9 days (no. of patients)	4	3	3.0 (1.3–6.9)	0.02
Median Apgar score at 1 min	7	8		0.04
Median Score for Neonatal Acute Physiology	12	9		0.001
Median duration of treatment (days)				
Total parenteral nutrition	20	19		0.05
Lipid emulsion	20	19		0.06
Antimicrobial agents	29	16		0.001
Exposure to Nurse A (no. of patients)	14	26	8.75 (1.2–62.5)	0.004
Exposure to Nurse B (no. of patients)	14	27	8.20 (1.1–58.5)	0.006

\*CI denotes confidence interval.

was performed from 50 percent of the time (reported by 1 respondent) to 100 percent of the time (reported by 48 respondents). The most common reasons for not washing hands 100 percent of the time were “baby needs immediate attention” (21 percent), “too busy” (15 percent), or “hands too chapped or dry” (7 percent). The distance between incubators and hand-washing facilities was adequate.

**TABLE 2.** RESULTS OF THE MULTIVARIATE ANALYSIS OF RISK FACTORS FOR *M. PACHYDERMATIS* COLONIZATION AND INFECTION AMONG PATIENTS IN THE INTENSIVE CARE NURSERY, OCTOBER 1993 THROUGH JANUARY 1995.

RISK FACTOR	ODDS RATIO (95% CI)*	P VALUE
Score for Neonatal Acute Physiology >9	19.7 (2.1–182.6)	0.01
Arterial catheterization for >9 days	29.5 (1.7–508.8)	0.02
Exposure to Nurse A	74.7 (2.7–2051.5)	0.01

\*CI denotes confidence interval.

The health care workers were observed to wash their hands before and after contact with patients significantly more frequently after in-service education and administration of the hand-washing questionnaire (after the intervention, procedures were correctly followed in 27 of 32 instances that should have involved hand washing, as compared with 15 of 46 before the intervention;  $P < 0.001$ ).

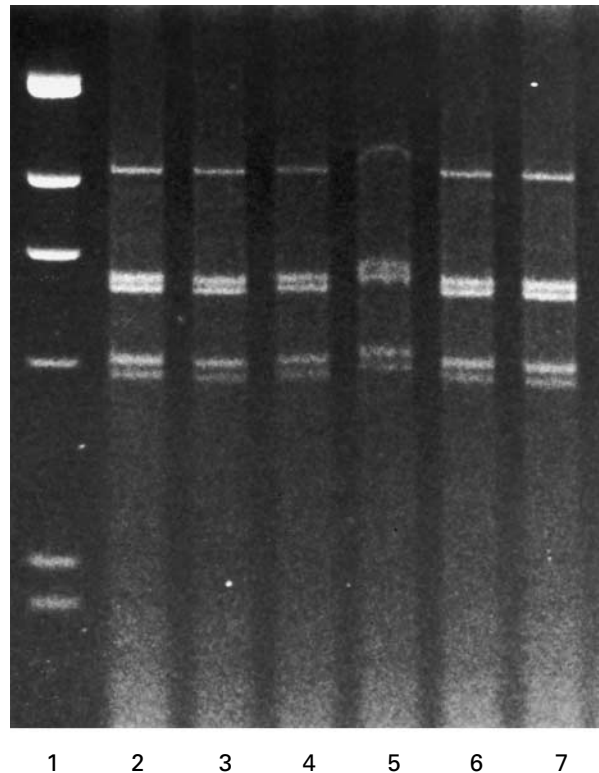
A point-prevalence survey of all infants and nurses on the unit at the change of shift on February 27, 1995, showed that in addition to the 15 case patients, 9 of 23 infants (39 percent) and 1 of 9 nurses (Nurse C) (11 percent) had positive cultures for *M. pachydermatis*. Twelve of 39 health care workers' pet dogs (31 percent) had positive cultures; all other pets had negative cultures.

**Molecular Typing**

Studies of restriction-fragment-length polymorphisms showed that *M. pachydermatis* isolates from all 15 case patients, Nurse C, and 3 of the 12 culture-positive dogs (25 percent) had the same banding pattern, associated with strain B (Fig. 2). The other nine culture-positive dogs had a variety of genomic patterns: strain A was identified in four isolates (33 percent), strain C in two (17 percent), and strain D in three (25 percent) (Fig. 3).

**Follow-up Studies**

Control measures implemented included increased education of health care workers about the *M. pachydermatis* outbreak and the importance of hand washing, and discharge of infants with *M. pachydermatis* colonization or infection after appropriate treatment. No additional cases of *M. pachydermatis* infection developed in the infants. A follow-up point-prevalence study of 21 patients and 58 health care workers in the intensive care nursery in June 1995, four months after the last case patient had been discharged, identified no cases of *M. pachydermatis* colonization.



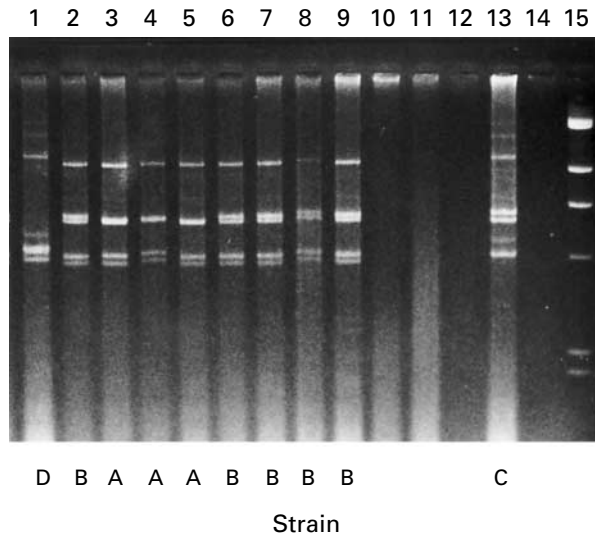
**Figure 2.** Pulsed-Field Gel Electrophoretic Patterns with Restriction Enzyme *HaeIII* in *M. pachydermatis* Isolates from Infants in the Intensive Care Nursery.

Lane 1 shows the molecular-size standards (lambda ladder), lane 2 an isolate from Baby 1's groin, lane 3 an isolate from Baby 1's face, lane 4 an isolate from Baby 2's groin, lane 5 an isolate from Baby 3's groin, lane 6 an isolate from Baby 3's perineum, and lane 7 an isolate from Baby 4's face. All lanes have identical banding patterns (*M. pachydermatis* strain B).

**DISCUSSION**

The malassezia genus is a group of lipophilic yeasts containing seven known species, including *M. furfur* and *M. pachydermatis*.<sup>9</sup> *M. furfur*, a common obligate saprophyte in humans, has caused nosocomial outbreaks in low-birth-weight infants receiving lipid-rich intravenous infusions in intensive care nurseries.<sup>10-13</sup> Skin colonization has been documented in patients and health care workers in intensive care nurseries, but the original source of *M. furfur* in these outbreaks was not identified.

*M. pachydermatis* is an emerging pathogen that has only relatively recently been reported to cause infection in humans.<sup>1,14</sup> It was first described in 1925 in a rhinoceros with exfoliative dermatitis<sup>15</sup> and was subsequently found to be associated with otitis media and otitis externa in dogs.<sup>16</sup> An outbreak of *M. pachydermatis* fungemia in patients in an intensive care nursery was traced to person-to-person transmission through the hands of health care work-



**Figure 3.** Pulsed-Field Gel Electrophoretic Patterns with Restriction Enzyme *Hae*III in *M. pachydermatis* Isolates from Infants in the Intensive Care Nursery and Health Care Workers' Dogs.

Lane 1 shows an isolate from Dog 1's ear, strain D; lane 2 an isolate from Dog 2's ear, strain B; lane 3 an isolate from Dog 3's ear, strain A; lane 4 an isolate from Dog 4's ear, strain A; lane 5 an isolate from Baby 5's groin, strain A; lane 6 an isolate from Baby 5's groin, strain B; lane 7 an isolate from Baby 1's groin, strain B; lane 8 an isolate from Baby 1's back, strain B; lane 9 an isolate from Baby 1's perineum, strain B; and lane 13 an isolate from Dog 5's ear, strain C. Lane 15 shows the molecular-size standards ( $\lambda$  ladder). Lanes 10, 11, 12, and 14 are empty.

ers.<sup>1</sup> However, the mechanism of introduction and the reservoir for the organism were not identified.

Risk factors for the transmission of *M. pachydermatis* are ill defined. In our study, low-birth-weight infants who were severely ill or had arterial catheters in place for more than nine days were at increased risk for colonization and infection. Unlike the findings in outbreaks caused by *M. furfur*, receipt of lipid infusions and exposure to other intravascular devices were not identified as risk factors. The difference in risk factors may be due to the fact that *M. furfur* is an obligate lipophilic organism whereas *M. pachydermatis* is not or to the fact that nearly all the infants in our cohort had intravascular catheters and received total parenteral nutrition and lipid emulsion.

Identification of a single clone (strain B) in all patients on the basis of restriction-fragment-length polymorphisms strongly supports patient-to-patient transmission of *M. pachydermatis* in this intensive care nursery. Health care workers were the most likely vector for transmission between patients. Although there is only one report of *M. pachydermatis* cultured from normal adult skin,<sup>17</sup> our culture survey in February 1995 documented an identical strain of *M. pachydermatis* in a culture specimen ob-

tained from Nurse C's hands while she was providing care in the nursery; a repeated culture in June 1995 was negative. (Nurse C reported no dermatologic problems other than dry skin.) These findings suggest that health care workers' hands can be transiently colonized with the organism. Hand colonization could have resulted in transmission to other patients in the intensive care nursery, particularly since hand washing after each contact with a patient was not performed consistently. Our culture survey in February 1995 identified nine additional asymptomatic infants with *M. pachydermatis* colonization. Such infants could serve as an ongoing reservoir for *M. pachydermatis*.

Although epidemiologic or culture studies suggested that contact with several health care workers played a part in the transmission of *M. pachydermatis*, only exposure to Nurse A remained a significant risk factor in the multivariate analysis. Nurse A, who had had documented contact with 14 of the 15 case patients, reported that she owned no pets and had no medical or skin problems other than dry skin. In the June point-prevalence study, the culture specimen from Nurse A was negative for *M. pachydermatis*. However, as shown with Nurse C, transient *M. pachydermatis* colonization can occur, with subsequently negative culture results.

The route by which *M. pachydermatis* was introduced into the intensive care nursery is not certain. It is possible that an infant became colonized during transit through the birth canal at delivery. Although there is a report of a positive vaginal culture,<sup>18</sup> this route seems unlikely in our outbreak, since the majority of the case patients were delivered by cesarean section, and none of those who were born of twin gestations had *M. pachydermatis* colonization or infected siblings (data not shown).

A more likely explanation is that one or more health care workers were vectors, introducing *M. pachydermatis* into the intensive care nursery. We hypothesize that colonization in the health care workers occurred by a transfer of the yeasts from pet dogs. This explanation is plausible because a large number of the health care workers owned dogs, a large number of the dogs were colonized with various strains of *M. pachydermatis*, and a common restriction-fragment-length polymorphic genotype caused colonization in the dogs, colonization and infection in the infants, and colonization in the health care workers. The three dogs with the pattern of restriction-fragment-length polymorphisms identified in the case patients were owned by three different health care workers. *M. pachydermatis* may have been introduced into the intensive care nursery on the hands of the health care workers, and hand colonization may have perpetuated the transmission of the organism from patient to patient within the nursery.

The most important limitation of our investiga-

tion is the small number of culture specimens obtained from the hands of health care workers while the organism was still present in the nursery. Although specimens were obtained from nine nurses, one third of the house officers, and several attending physicians in February 1995, further cultures were delayed until the results of the earlier culture and genotype studies were available.

In this outbreak, observational, culture, and genotyping studies strongly suggest that *M. pachydermatitis* was transmitted from patient to patient on the hands of health care workers. In June 1995, after hand-washing practices had been improved, all cultures from the nursing staff and attending physicians were negative for *M. pachydermatitis*. The organism was no longer being transmitted to the infants, and the outbreak had ceased. Careful hand washing by health care workers on reporting to work and before and after all contacts with patients is essential to prevent the introduction and subsequent nosocomial transmission of pet-associated pathogens, such as *M. pachydermatitis*, in the intensive care nursery.

*We are indebted to Jerome I. Tokars, M.D., M.P.H., for statistical help.*

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