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Survival of Airborne Mycoplasma as Affected by Relative Humidity

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The cause of death of aerosolized bacteria is unclear. Most hypotheses favor dehydration with accompanying intracellular molecular rearrangement, concentration of toxic products, or imbalance of metabolic functions as the primary lethal effect (R. J. Zentner, Bacteriol. Rev. **30:** 551, 1966). Since most bacteria studied in the aerosol state show similar responses to aerosol stress (J. D. Anderson and C. S. Cox, Airborne Microbes, p. 203, London, 1967), and in order to clarify the cause of microbial aerosol death, an effort was made to find a microorganism significantly different from bacteria, which could be studied as an aerosol.

Mycoplasmas were chosen for this study because of their unique physiological and morphological characteristics. These organisms, which have minimal reproductive units approximately 125 mμ in diameter, are the smallest living forms that can be cultured in a cell-free system. They have no rigid cell wall as do the bacteria, but are bound solely by a sterol-containing triple-layered unit membrane 75 to 100 A in thickness (C. H. Domermuth et al. J. Bacteriol. **88:**727, 1964). In addition, our knowledge of the epidemiological role of airborne pathogenic mycoplasmas is very limited. Few mycoplasmas have been studied as aerosols (R. B. Kundsin, J. Bacteriol. **91:**942, 1966; C. W. Beard and D. P. Anderson, Avian Diseases **11:**54, 1967) and none over a wide relative humidity (RH) range. Therefore, by examining the effect on airborne mycoplasmas of various humidity levels over extended periods of time, we hoped to gain an increased understanding of their role as possible airborne contagion and the effects of RH upon the survival of such contagion.

Mycoplasma laidlawii B and *M. gallisepticum* strain S6 were grown in the medium of Shklair et al. (J. Bacteriol. **83:**785, 1962) modified to contain 10% inactivated horse serum and 2% yeast autolysate. Growth medium containing 1.4% agar was used to culture organisms recovered from the aerosol state. Penicillin (50 units/ml) and amphotericin B (5 μg/ml) were added to the medium to control bacterial and fungal contaminants.

Cultures in the stationary phase of growth, which contained between 10^8 and 10^9 organisms per milliliter, were aerosolized with a modified Wells reflux atomizer into two 500-liter rotating drums (L. J. Goldberg et al. Am. J. Hyg. **68:**85, 1958) held at 27 C. The RH in these drums was controlled by mixing wet and dry air in the secondary airstream and was measured with a wet and dry bulb thermometer. Physical decay of the aerosol was determined by measuring the relative light scatter of the aerosol with a forward-angle light-scatter photometer.

The cultures were atomized for 10 min, followed by a 5-min equilibration period to allow uniform dispersal of the aerosol. Samples were then collected at intervals over a 5-hr period, by use of all-glass capillary impingers containing PPLO broth (Difco). The number of colony-forming units (CFU) was determined by plating serial dilutions of the impinger broth.

Figure 1 shows the survival of these organisms in aerosols as a function of RH. Surviving organisms are shown as a fraction of the initial recovery (CFU recovered immediately after equilibration). *M. laidlawii* was very stable at humidity levels of 25% or lower and at 75% or higher. However, the number of CFU decreased rapidly when this organism was exposed to mid-range humidities, e.g., at 40% RH, only 1% of the organisms initially present was recovered after 5 hr.

In general, *M. gallisepticum* in the airborne state did not survive well except at 10% RH. At least 90% of the organisms were lost after 5 hr at RH values of 25% or greater, and at 50 and 60% RH no organisms were recovered in the initial sample, thus showing the extreme sensitivity of *M. gallisepticum* to these relative humidities.

Our data showed a greater decay rate for *M. gallisepticum* at mid-range (40 to 60%) humidities than that reported by Beard and Anderson (Avian Diseases **11:**54, 1967), who demonstrated

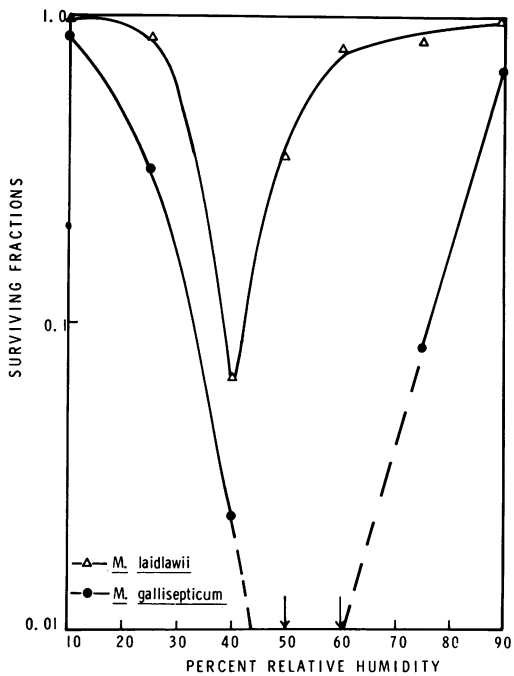

FIG. 1. *Effect of relative humidity on survival of Mycoplasma species laidlawii and gallisepticum in aerosols at 27 C for 60 min. Data shown are corrected for physical loss and represent biological decay. This figure is taken from decay-rate values obtained by best fit techniques, representing four or six experiments at each relative humidity. No single value deviated more than 10% from those shown.*

a 99% loss of viability after 6 hr. Therefore, we conducted additional studies with the dynamic aerosol transport apparatus (DATA) of Hatch and Dimmick (Bacteriol. Rev. **30**:597, 1966), which can be used to measure the survival of microbial aerosols at ages from 15 sec to about 15 min, in increments of 30 sec. DATA experiments with *M. gallisepticum* at 40, 50, and 60% RH showed that the greatest decrease in survival was during the first 5 min, with 99% of the organisms not recoverable after this time. These data support our results obtained with the use of the rotating drum.

Dunklin and Puck (J. Exptl. Med. **87**:87, 1948) demonstrated that the survival of airborne bacteria is affected by RH; and these workers, as well as Beebe (J. Bacteriol. **78**:18, 1959), showed that midrange RH's were the most lethal for certain bacterial species. Our studies with mycoplasma showed results similar to those for bacteria, as the mid-range RH levels were most lethal. Although bacteria and mycoplasma differ greatly in cell size, composition, and morphology of cell boundary, their similarity in response to aerosolization throughout a range of RH values suggests that the mechanism of death of microorganisms in aerosols is independent of these unique features and may be similar for both bacteria and mycoplasma.

It is further shown that the viability of mycoplasma at optimal humidity levels is sufficient to provide long-term animal infectivity of aerosols if virulence is retained in the airborne state. Although *M. gallisepticum*, the etiological agent of chronic respiratory disease of poultry, is largely transmitted by transovarian passage (H. van Roekel et al., Am. J. Vet. Res. **19**:453, 1958), airborne infection can and does occur (L. C. Grumbles et al., Poultry Sci. **31**:309, 1952). These infections indicate that virulence of airborne mycoplasma can be retained under some conditions, whereas the data reported here suggest that a measure of disease control among breeder flocks may be accomplished by control of atmospheric RH.

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