Survival of Airborne *Pasteurella tularensis* at Different Atmospheric Temperatures

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The aerosol survival, recovery, and death rate of *Pasteurella tularensis* SCHU S5 disseminated in particle sizes of 1 to 5 μm were significantly affected by air temperature. The highest aerosol recovery of viable *P. tularensis* was observed within −7 and 3 C; the recovery decreased significantly below and above this temperature range. The death rate of airborne *P. tularensis* was not significantly influenced by an increase in temperature from −40 to 24 C. However, a progressive increase in atmospheric temperature from 24 to 35 C resulted in increased death rates; thus, a linear relationship appeared to be present between the temperature and death rates. At 49 C, the recoveries of viable airborne *P. tularensis* were significantly lower and the death rates were higher than at the other temperatures.

The knowledge of aerosol behavior of infectious microorganisms is important to the understanding of the transmission of diseases by the respiratory route. Such knowledge is also essential in experimental studies in which a respiratory challenge of animals with a well-defined inhaled dose of airborne microorganisms is desired. Recent reports have shown that the survival of airborne *Pasteurella tularensis* is significantly influenced by relative humidity (RH; 2, 3). Maximal aerosol survival of *P. tularensis* disseminated as a wet preparation was observed at humidities above 75% RH, and minimal survival occurred between 30 and 60% RH.

Although a considerable amount of information is available pertaining to the effect of various humidities on the survival and infectivity of airborne *P. tularensis*, the aerosol behavior of this organism at various atmospheric temperatures has not been reported. The studies summarized in this paper were designed to examine the death rates and aerosol survival of *P. tularensis* at atmospheric temperatures ranging from −40 to 49 C.

**MATERIALS AND METHODS**

Concentrated stock cultures of streptomycin-resistant *Pasteurella tularensis* strain SCHU S6 and frozen concentrated slurries of spores of *Bacillus subtilis* var. *niger* (batch 91) were provided by the U.S. Army, Fort Detrick. *P. tularensis* was grown in phosphate-buffered N-Z amine A medium, containing glucose, cysteine, and thiamine (7). After 14 h of incubation, the harvest was concentrated approximately 10-fold by centrifugation. This provided a stock culture containing about 150 × 10^5 viable *P. tularensis* cells/ml. Stabilizing agents added to the culture were 5% raffinose and 0.1% dipyridyl (7). The *B. subtilis* spores, used in the aerosol experiments as the physical decay tracer, were stored at dry ice temperature until used. *B. subtilis* slurries were prepared by mixing 11.5 g of the frozen spores with 100 ml of sterile gelatin-phosphate diluent (0.2% gelatin-0.4% NaHPO₄, at pH 7.0). The suspensions were then briefly blended in a Waring Blendor, filtered through an 80-mesh stainless-steel screen, and stored at 4 C. Stock cultures of *P. tularensis* were stored at 4 C and used for the aerosol experiments within less than 4 weeks from receipt. For dissemination, fresh mixtures were prepared daily by adding the *B. subtilis* suspension to the stock culture of *P. tularensis* to provide a 10:1 ratio of the vegetative bacteria to the spore tracer.

The methodology used to establish and maintain the desired environmental conditions in the 2,500-liter gas-tight stainless-steel aerosol chamber and the techniques of handling the infectious materials in the microbiological safety cabinet system (class III) were the same as previously described (4, 9). The mixtures of the organisms were disseminated by means of a two-fluid atomizer (FK-8 aerosol gun), which produced aerosol clouds with a majority of particles in the 1- to 5-μm range (6). The temperature conditions in the aerosol chamber ranged from −40 to 49 C, and the humidity, from 85 to 99% RH.

The aerosol in the chamber was sampled for 1 min at ages of 4, 16, 32, and 64 min with two parallel all-glass impingers (AGI-30) operated at a sampling rate of 12.5 liters per min (1). A single-stage impactor designed to remove particles larger than 5 μm re-
placed the conventional curved stem of the AGI-30 (8). The impingers contained 20 ml of sterile gelatin-saline (0.1% gelatin at pH 7.0) collecting fluid with 0.15% Dow-Corning antifoam A emulsion. Freezing of the sampling fluid during experiments at the sub-freezing temperatures was prevented by keeping the AGI-30 samplers in a water bath at approximately 20 C.

Contents of the duplicate impingers were pooled for the quantitative assay of the airborne microorganism. After appropriate dilutions were prepared in gelatin-saline, *P. tularensis* was assayed on glucose cysteine blood agar plates incubated for 72 h at 37 ± 1 C. *B. subtilis* was assayed conventionally on tryptose agar (5).

The death rates of *P. tularensis* were estimated by first quantitating the numbers of viable airborne microorganisms per unit of air volume at 4, 16, 32, and 64 min after dissemination and converting the numbers to percent recoveries. These values expressed the recovery of the microorganisms at the four sampling periods as a percentage of the microorganisms present in the original suspension used for dissemination. When plotted, the slopes of the resulting curves, expressed in percent per minute (%/min) units, represented the total aerosol decay rate over the 64-min cloud age. This decay rate included losses due to physical factors and those due to death of the microorganisms. The physical decay rates of the aerosol, which defined the losses of aerosol concentration due to factors other than death of the microorganisms (e.g., settling of particles and impingement on the chamber walls), were determined on the basis of recoveries of *B. subtilis* spores disseminated as an intimate mixture with *P. tularensis*. The differences between the total decay rates and the physical decay rates provided estimates of the biological decay rates (death rates) of *P. tularensis*.

Estimates of the percent survival provided another means of evaluating *P. tularensis* viability in aerosols. The percent survival was calculated on the basis of the ratio between *P. tularensis* and *B. subtilis* spores initially present in the dissemination mixture, representing 100% recovery, and the ratio of the two microorganisms in aerosol samples collected at the various cloud ages.

The mean aerosol recoveries and death rates were based on results of a minimum of six replicate trials conducted for each experimental condition. Whenever applicable, the means were compared by a standard analysis of variance, and the significance of the observed differences was reported at the 5% probability level.

**RESULTS AND DISCUSSION**

The specific temperature conditions investigated were -40, -29, -18, -7, 0, 3, 24, 29, 35, and 49 C. In trials conducted within the temperature range of -3 to 35 C, the humidity was maintained at 85 ± 5% RH. At the lower temperatures (-7 to -40 C), the humidity in the aerosol chamber was ambient and represented essentially saturated atmosphere. During experiments conducted at 49 C, the humidity was maintained at both 70 and 90% RH.

Table 1 shows the mean death rates based on 64-min aerosol age and early recoveries of *P. tularensis* disseminated at -40 to 35 C. The observed differences among the death rates of *P. tularensis* within the -29 to 3 C temperature range were not significant. The mean death rate calculated over the six temperature conditions was 1.3%/min. Within the temperature range of 24 to 35 C, the mean death rate of the microorganisms was 5.3%/min, and the differences among the three death rates were significant. Furthermore, the relationship of *P. tularensis* death rate to temperature was essentially linear: increasing environmental temperature resulted in increased death rates. In all experiments, the decay rates of *B. subtilis* spores were not significantly influenced by the environmental temperature, in confirmation of previously reported findings (5).

Total percent recoveries of viable *P. tularensis* at the 4-min sampling period are also shown in Table 1. These values express the recovery of *P. tularensis* at 4 min as a percentage of the concentration of this microorganism disseminated from the original suspension. The relatively low overall percent recoveries of the *P. tularensis* at this sampling period primarily reflect the losses of viable bacteria due to the stress of dissemination. The highest recovery of viable *P. tularensis* was obtained within the -7 to 3 C temperature range, with a mean of 8.3% calculated over the four experimental conditions. The recoveries decreased significantly below and above this temperature range. Within the -18 to -40 C range, the mean aerosol recovery of viable *P. tularensis* was 3.4%,

### Table 1. Recovery and death rate of airborne *P. tularensis* at various temperatures

| Temp (C) | RH (%) | Aerosol parameter | Death rate (%/min) | Recovery (%) |
|----------|--------|-------------------|--------------------|--------------|
| -40      | Ambient|                   | 2.4                | 2.6          |
| -29      | Ambient|                   | 1.9                | 4.1          |
| -18      | Ambient|                   | 0.8                | 3.6          |
| -7       | Ambient|                   | 1.9                | 7.0          |
| -3       | 85     |                   | 1.3                | 8.7          |
| 0        | 85     |                   | 1.2                | 10.1         |
| 3        | 85     |                   | 0.7                | 7.3          |
| 24       | 85     |                   | 2.9                | 3.1          |
| 29       | 85     |                   | 4.3                | 1.9          |
| 35       | 85     |                   | 8.7                | 0.92         |

* RH* Relative humidity.
* Death rate based on 64-min cloud age.
* Percent viable recovery at 4-min cloud age.
and for 24, 29, and 35 C the mean recovery was 2.0%. Within the 24 to 35 C range, the relationship between recoveries and air temperature was linear, similar to that observed for death rates. Increasing chamber temperature resulted in decreased recoveries of airborne <i>P. tularensis</i>. The data showing the high recovery within the range of −7 to 3 C were obtained in four separate experiments in which different preparations of <i>P. tularensis</i> were used. Therefore, it appears that the high recovery of viable <i>P. tularensis</i> at 4-min cloud age within this temperature range represents a true and reproducible occurrence.

The percent survival of <i>P. tularensis</i> as a function of aerosol age is plotted for various temperatures in Fig. 1. This representation confirms the relationship between the survival and death rates of airborne <i>P. tularensis</i> and the environmental temperature.

The reduced survival of <i>P. tularensis</i> at −40 C is a phenomenon which was observed during studies of other airborne vegetative microorganisms, namely, <i>Serratia marcescens</i> and <i>Escherichia coli</i> (5). The factors responsible for this reduced aerosol survival appear to include, in part, the physical characteristic of the slurry and the configuration of the particles upon dissemination at the very low temperature and saturated atmosphere.

Recovery of viable <i>P. tularensis</i> was significantly lower at 49 C than at the other temperatures. The estimated death rates of <i>P. tularensis</i> at 49 C ranged from 12.0 to 13.3%/min. At 4 min after dissemination, 7,900 and 800 organisms per liter of air were recovered at 70 and 90% RH, respectively, compared to 20.7 × 10⁴ and 18.8 × 10⁴ organisms per liter of air at 24 C and the two humidity conditions. At a cloud age of 64 min, the recovery was 500 and 80 viable <i>P. tularensis</i> cells per liter of air at 49 C and 70 and 90% RH, respectively, as compared to 16.6 × 10⁴ and 18.1 × 10⁴ organisms per liter of air at 24 C.

An awareness and knowledge of the relationship between temperature and survival of airborne infectious microorganisms is an important factor in experimental studies of infections involving respiratory challenges. The estimation of inhaled doses is dependent on the respiratory tidal volume of the host animal, the number of viable organisms per liter of air, and the duration of exposure to the aerosol. If the initial aerosol concentration, the death rate, and the approximate lethal dose (LD₅₀) of the infectious agent are known, the duration of exposure to the aerosol that is required to obtain a given fractional response can be closely estimated. For example, assuming an equivalent initial aerosol concentration of <i>P. tularensis</i>, after 60 min the inhaled aerosol would contain approximately 4, 75, and 1,500 times more viable <i>P. tularensis</i> cells at 24 C than at 29, 35, and 49 C, respectively. Accordingly, the effect of temperature on the decay rate of <i>P. tularensis</i> could significantly skew the expected mortalities in experiments involving animal exposures.

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