A Mouse Model for Estimation of *Pasteurella haemolytica* Deposition in Calf Lungs Following Aerosol Exposure

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ABSTRACT

A method used to calculate the number of *Pasteurella haemolytica* reaching the lungs of calves during an aerosol exposure is described. This method is based on a linear relationship of bacterial deposition in lungs of mice and calves when exposed to the same bacterial aerosol.

RÉSUMÉ

Cet article décrit une méthode utilisée pour calculer le nombre de *Pasteurella haemolytica* qui atteignent les poumons de veaux, au cours d'une séance de nébulisation. Cette méthode s'appuie sur une relation linéaire de déposition bactérienne dans les poumons de souris et de veaux, soumis au même aérosol bactérien.

INTRODUCTION

Infectious disease of the respiratory system, particularly pneumonic pasteurellosis (shipping fever), is one of the most important causes of morbidity and mortality of cattle (1,3,8). Pneumonic pasteurellosis is not a single entity but a complex host-bacteria-environment interaction, thus making experimental reproduction of the disease difficult (4,9). Although aerosol inoculation of *Pasteurella haemolytica* probably mimics most closely the "normal" route of infection, aerosolization has the major disadvantage that the numbers of *P. haemolytica* reaching the lung are unknown or else expensive to determine by the use of control animals (2,6). In this paper we describe a simple and inexpensive method for determination of the numbers of *P. haemolytica* that reach the bovine lung without having to kill control (zero hour) calves. This method is based on the observation that there is good correlation in the numbers of bacteria deposited in lungs of mice and calves when these animals are exposed to the same aerosol of bacteria (2,6).

MATERIALS AND METHODS

The aerosol apparatus used in this experiment is similar to that designed by Laurenzi *et al* (5), with modifications (2,6,7). Fourteen different trials were conducted in which five white mice, weighing about 30 g each, together with one Holstein-Friesian calf, were exposed to an aerosol of *P. haemolytica*. The calves were eight to 16 weeks old. In brief, the heads of calves protruded into, and caged mice were contained within, a plexiglass chamber through which was drawn air containing an aerosol of microbes. The aerosol was generated by glass nebulizers under compressed air pressure. The particle size produced by the nebulizers ranged from 1 to 5 μm. A range of 10^8 to 10^{10} organisms in 150 mL of medium was aerosolized in each trial. Following exposure to an aerosol of *P. haemolytica* for 30 minutes, the calf and mice were killed immediately. Time of death is referred to as zero hour. The techniques involved in euthanasia and collection of samples are described in detail elsewhere (2,6,7).

The numbers of *P. haemolytica* in the lungs of mice were determined by homogenizing the lungs of each mouse in 5 mL of phosphate-buffered saline solution (PBS) (0.1 M; pH 7.3-7.5), and plating in triplicate serial tenfold dilutions from 1 to 10^{-4} on blood agar. The plates were incubated at 37°C for 12-14 hours and the number of colonies counted on all plates (2). The number of bacteria per gram of mouse lung was calculated according to the following formula:

$$\text{No. of bacteria per g of mouse lung} = \frac{\text{No. of colonies} \times 5.0 \times \text{dilution factor} \times 4}{0.24}$$

where:

No. of bacteria = Number of *P. haemolytica* deposited in mouse lungs at zero hour

No. of colonies = Total number of colonies present on the three plates at a countable dilution

5.0 = Number of mL of PBS added to mouse lung for grinding purposes

4 = Transformation factor for 1 g of mouse lung. (The average lung

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weight for a 30 g mouse is 250 mg; therefore 4 × 250 mg = 1 g of mouse lung.)

0.24 = The number of mL of the lung homogenate plated on the media (12 drops of 0.02 mL = 0.24 mL)

Dilution Factor = Factor of dilution of the lung homogenate from the countable plate dilution used above

The mean number of bacteria in one gram of mouse lung was determined for each group of five mice. This mean was compared with the number of bacteria in the calf lung.

The number of *P. haemolytica* in bovine lung was determined by homogenizing six samples of lung per calf [center of lateral aspect of left and right apical (cranial), middle (cardiac), and diaphragmatic (caudal) lobes]. Each sample weighed 10 g and was homogenized in 100 mL of PBS. The homogenates were diluted and plated similarly to the homogenates of normal lung. The number of bacteria per gram of bovine lung was calculated from the following formula:

$$\text{No. of bacteria in one g of calf lung} = \frac{\text{No. of colonies} \times 10 \times \text{dilution factor}}{0.24}$$

where:

No. of bacteria = Number of *P. haemolytica* present in calf lung

No. of colonies = Total number of colonies present on three plates at a countable dilution

10 = Number of mL of PBS added to calf lung for grinding purposes

Dilution Factor = Factor of dilution of the lung homogenate from the countable dilution used above

0.24 = Number of mL of lung homogenate plated on the media (12 drops of 0.02 mL = 0.24)

The arithmetic mean of the bacteria in the six lung samples per calf was determined and was compared with the mouse value.

RESULTS

The number of bacteria present per gram of mouse lung will be represented hereafter by X and the number of bacteria per gram of bovine lung by Y. Thus, each of the 14 trials has one value for X (mean number of bacteria per gram of lung in five mice) and a corresponding value for Y (mean number of bacteria per gram of calf lung). The mean bacterial counts for X in the 14 trials ranged from 10^4 to 10^7/g with an overall mean of 7.44×10^6/g. The mean bacterial count for each calf (Y) ranged from 10^4 to 10^7/g with an overall mean of 3.08×10^6/g (Table I).

TABLE I. *P. haemolytica* Deposited Per Gram of Lung in Mice (X) and Calves (Y)

Experiment	X	Y
1	3.47×10^6	4.99×10^5
2	1.43×10^6	4.87×10^5
3	3.32×10^7	1.36×10^7
4	1.89×10^7	7.06×10^6
5	3.12×10^6	5.72×10^6
6	9.80×10^6	6.70×10^6
7	1.53×10^7	5.98×10^6
8	8.54×10^4	1.06×10^4
9	1.21×10^6	4.14×10^5
10	3.89×10^6	5.35×10^5
11	3.68×10^6	5.08×10^5
12	9.16×10^5	4.90×10^5
13	3.94×10^6	1.08×10^6
14	4.92×10^4	1.08×10^4
Mean	7.44×10^6	3.08×10^6

The null hypothesis, that retention of *P. haemolytica* in mouse and calf lungs was not correlated ($H_0: \rho = 0$), was rejected ($p < 0.001$, $t = 12.04$, $df = 12$), and the correlation coefficient (r) of 0.961 indicates a strong correlation between X and Y values. The regression coefficient (B) is 0.419 with a standard error of 0.035, and the Y intercept (A) is -38222 (Fig. 1). From this regression the numbers of *P. haemolytica* deposited in one gram of calf lung can be calculated from values obtained in one gram of mouse lung by the equation $Y = A + BX$, where Y = numbers of *P. haemolytica* in one gram of bovine lung, and X = numbers of *P. haemolytica* in one gram of mouse

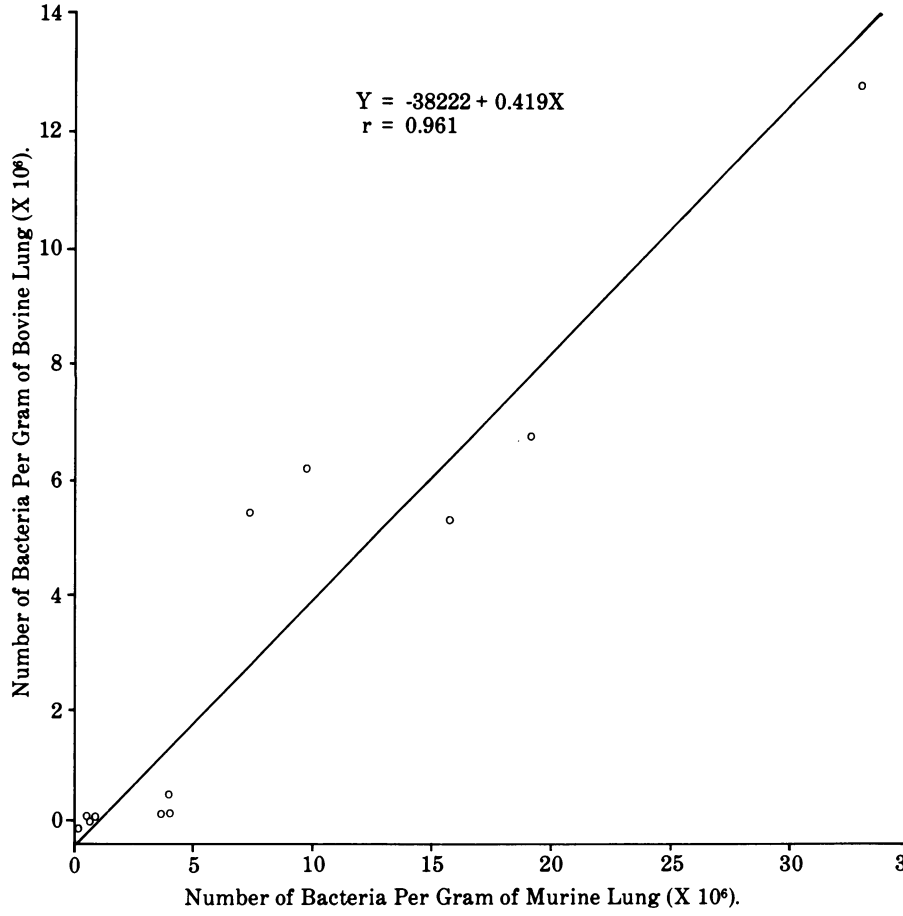

Fig. 1. Deposition of *Pasteurella haemolytica* in the lungs of mice and calves exposed to the same bacterial aerosol.

lung. Thus the formula used to calculate the values for calves is: Y = 38222 + 0.419X, when both calves and mice are exposed to the same aerosol of *P. haemolytica*.

DISCUSSION

Our results demonstrated that there was a direct linear relationship between numbers of *P. haemolytica* retained in the lungs of mice and calves following exposure to the same aerosol for 30 minutes. It should be understood that this particular regression may apply only to our aerosol apparatus and our laboratory conditions, and the regression could be altered by changes in aerosol particle size or by altering the duration of exposure from the 30 minutes which we used. Other researchers can similarly establish the correlation that exists between the number of bacteria in the lungs of calves and mice for their types of bacterial aerosol, aerosol apparatus, and environmental conditions. This method permits extrapolation to zero time concentration of *P. haemolytica* in the bovine lung based on a simultaneous mouse exposure, and allows calculation of clearance of *P. haemolytica* from the bovine lung at various times postaerosolization, or alternatively, calculation of retention of *P. haemolytica* by the bovine lung. This technique has been used in several experiments in which alterations of clearance were measured (2,6,7).

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REFERENCES

1. **BRYSON, D.G., J.B. McFERRAN, H.J. BALL** and **S.D. NEILL.** Observations on outbreaks of respiratory disease in housed calves. 1. Epidemiological, clinical and microbiological findings. Vet. Rec. 103: 485-489. 1978.
2. **GILKA, F., R.G. THOMSON** and **M. SAVAN.** The effect of edema, hydrocortisone acetate, concurrent viral infection and immunization on the clearance of *Pasteurella hemolytica* from the bovine lung. Can. J. comp. Med. 38: 251-259. 1974.
3. **JENSEN, R., R.E. PIERSON, P.M. BRADDY, D.A. SAARI, H. LEVERMAN, J.J. ENGLAND, D.P. HORTON** and **A.E. McCHESNY.** Disease of yearling feedlot cattle in Colorado. J. Am. vet. med. Ass. 169: 497-499. 1976.
4. **JERICHO, K.W.F.** Update on pasteurellosis in young cattle. Can. vet. J. 20: 333-335. 1979.
5. **LAURENZI, G.A., L. GERMAN, M. FIRST** and **E.H. KASS.** A quantitative study of deposition and clearance of bacteria in the murine lung. J. clin. Invest. 43: 759-768. 1964.
6. **LILLIE, L.E.** and **R.G. THOMSON.** The pulmonary clearance of bacteria by calves and mice. Can. J. comp. Med. 36: 129-137. 1972.
7. **LOPEZ, A., R.G. THOMSON** and **M. SAVAN.** The pulmonary clearance of *Pasteurella haemolytica* in calves infected with bovine parainfluenza 3 virus. Can. J. comp. Med. 40: 385-391. 1976.
8. **MARTIN, S.W., A.H. MEEK, D.G. DAVIS, R.G. THOMSON, J.A. JOHNSON, A. LOPEZ, L. STEPHENS, R.A. CURTIS, J.F. PRESCOTT, S. ROSENDAL, M. SAVAN, A.J. ZUBAIDY** and **M.R. BOLTON.** Factors associated with mortality in feedlot cattle: The Bruce County beef project. Can. J. comp. Med. 44: 1-10. 1980.
9. **THOMSON, R.G.** A perspective on respiratory disease in feedlot cattle. Can. vet. J. 21: 181-185. 1980.