Deposition in the Respiratory Tract of Cattle of Spores of Bacillus subtilis var niger by Inhalation and by Nasal Instillation

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ABSTRACT

Spores of Bacillus subtilis var niger were deposited in the lungs, tracheae and nasal cavities of four calves by aerosol inhalation and in three calves by intranasal instillation. From each calf 20 specimens of lung tissue, each weighing one gm, three of trachea and three of nasal mucosa were examined for spore content. The average numbers of spores per gm of lung tissue from animals exposed to aerosols were 3.05 and 4.84, 2.35 and 2.02 x 10^4. Lungs from animals exposed intranasally contained only 747, 62 and 1424 spores per gm of tissue respectively. Animals exposed intranasally had a hundred to a thousand fold more spores on nasal mucosa than animals exposed by aerosol and the latter had a thousand fold more spores on tracheal mucosa than calves exposed intranasally. Aerosol inhalation exposed the lung and trachea more densely and uniformly than did intranasal instillation.

RÉSUMÉ

Cette expérience visait à introduire des spores de Bacillus subtilis var niger dans les poumons, la trachée et la cavité nasale de quatre veaux, au moyen de l'inhalation d'aérosols, et dans les mêmes tissus de trois autres, par l'installation intra-nasale. On procéda ensuite à la recherche de spores, chez tous ces veaux, par l'examen de 20 échantillons de tissu pulmonaire d'un poids individuel de 1g, trois de la trachée et trois de la muqueuse nasale. Le nombre moyen de spores par gramme de tissu pulmonaire, chez les veaux assujettis aux aérosols s'établissait respectivement à 3.05, 4.84, 2.35 et 2.02 x 10^4. Les poumons de ceux qu'on avait soumis à une instillation intra-nasale ne contenaient respectivement que 747, 62 et 1424 spores par gramme de tissu. Leur muqueuse nasale recelait cependant de 100 à 1,000 fois plus de spores que celle des veaux assujettis aux aérosols. La muqueuse trachéale de ces derniers contenait cependant 1,000 fois plus de spores que celle des veaux soumis à une instillation intra-nasale. L'inhalation d'aérosols ensemença la trachée et les poumons d'une façon plus intense et plus uniforme que ne le fit l'instillation intra-nasale.

INTRODUCTION

Experimental aerosol exposure of calves to infectious agents has been reported for the agents of infectious bovine rhinotracheitis (IBR) (33), *Myxovirus SF-4* (32), foot and mouth disease (19), rinderpest (29), *Mycoplasma mycoides* (18) and *Staphylococcus aureus* (21). Buffaloes have been similarly infected with *Pasteurella multocida* (28). Intranasal instillation of inocula into cattle has been used frequently for vaccine trials with IBR virus (24, 35), parainfluenza-3 virus (14, 25), *Mycoplasma mycoides* var *mycoides* (20) and in attempts to produce respiratory disease with bovine adenovirus (5, 6) reovirus (27) and parainfluenza-3 virus (3, 4, 7). The production of immunoglobulins locally

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after "aerial" vaccination of tissue of the upper respiratory tract particularly with antigens of myxovirus has received much attention (8, 11, 22, 34, 36).

Sites of critical resistance to infection have been recognized in the lungs (13) and these localities may vary for differing diseases (16). The exposure and response of these sites to infectious agents in aerosol is influenced by the particle size of the aerosol (1). Differing pathological conditions have been produced with aerosols of the same inocula (9, 10) but of differing particle size. Similarly, the method of respiratory tract exposure has been found to influence the pathogenesis of avian infectious laryngotracheitis (30). The aerosol method evoked extensive lesions throughout the respiratory tract, whereas the intranasal route confined lesions to the nasal cavity.

To ensure uniform response an experimental challenge by the respiratory route should enable microorganisms of inocula to reach all critical sites under study. It was therefore thought worthwhile to compare in lungs, trachea and nasal cavities the deposition and concentration of spores of the same inoculum, administered intranasally to calves or inhaled as aerosols.

MATERIALS AND METHODS

Suspensions of heat-shocked spores of *Bacillus subtilis* var *niger* (Bg), a harmless and stable bacterial agent, were prepared in distilled water at concentrations of 3.45 and 2.55 x 10^9 per ml. Aliquots of these suspensions were sprayed from a Collison atomizer in a modified Henderson apparatus[1] (17) to produce aerosols of 8.0 x 10^5 (high — H) and 6.3 x 10^5 (low — L) spores per liter of air respectively in an animal exposure device (2) with a flow rate of 155 liters of air per minute. The atomizer produced particle sizes with mass median diameters of two to three microns (23).

Two Hereford-cross calves (nos. 1H, 2H) were exposed to the high and two calves (nos. 3L, 4L) to the low aerosol concentrations for five minutes at temperatures of ten and 14°C and relative humidities of 39 and 60% respectively. During the exposure periods the rates of respiration were recorded. To estimate the five minute respiratory volume for each calf exposed by aerosol the respiratory rates were multiplied by the tidal volumes of similar sized calves (15). Based on the concentration of spores in the aerosols the number of spores inhaled by each calf was then estimated.

Three other comparable animals (#5N — 204 kg, 6N — 227 kg, 7N — 204 kg live weight) received intranasally (N) 2 ml of a spore suspension at a concentration of 3.45 x 10^9/ml. One ml was sprayed into each nostril by means of a syringe joined to a three-hole cannula supplied with a commercially available IBR vaccine[2]. (The terminal three holes of the cannulae varied greatly as to size and shape and some were partly occluded due to irregular molding.)

Immediately after the exposure by either means the animals were shot in the cranium and the lungs and trachea were removed from the animals within 15 minutes of death. The left major bronchus was transected and the left lung used for microbiological assay. Pieces of lung tissue in excess of one gm in weight were taken aseptically from beneath visceral pleura (1-2 cm), placed in individual petri dishes and frozen immediately. Five pieces were taken from each of the anterior lobe, the cardiac lobe and from the anterior and posterior segments of the diaphragmatic lobe. Their order was in a ventral to dorsal progression, with numbers 1, 6, 11 and 16 being at the ventral borders and numbers 5, 10, 15 and 20 at the dorsal borders of the respective lobes.

From calves 3L, 4L, 5N, 6N and 7N in addition to the collection of the lung tissues, mucosal samples 2.5 cm^2 in size were taken from the following sites: 5 cm distal to the larynx, 5 cm proximal to the apical bronchus and 5 cm distal to the first bronchial bifurcation and from anterior and posterior sites of ventral turbinate and posterior floor of the right nasal cavity (26).

Each piece of frozen lung tissue was thawed, trimmed to weigh 1 gm, ground

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up with the aid of sterile sand and made into an emulsion in 4 ml of distilled water. The pieces of mucosa (2.5 cm^2) were similarly treated but failed to emulsify. Samples of the aerosols to which the four calves were exposed were collected for the duration of exposure in distilled water in all glass impingers (AGI 6/15)[3]. Spore counts were determined (31) on the fluids of impingers, on suspensions used for aerosols and nasal sprays and on suspensions of tissues from exposed animals. The diluted suspensions were plated on tryptose agar.

The geometric means of spore counts for the lobes of all lungs exposed to aerosol were compared for significant differences using a paired t test (5%).

RESULTS

The data pertaining to body weight of calves, their number of respirations and total volumes inhaled during the aerosol exposures, spores inhaled per kg of body weight and the meteorological conditions at the times of the exposures are shown in Table I. Regional deposition of spores in the respiratory tracts differed significantly between aerosol or intranasally exposed calves. The average number of spores per gm of lung tissue from animals exposed to aerosols was 3.05 and 4.84, 2.55 and 2.02 x 10^4. Lungs from animals exposed intranasally contained only 747, 62 and 1424 spores per gm of tissue, respectively (Table II). Animals exposed intranasally had a hundred to a thousand fold more spores on tracheal mucosa than exposed intranasally (Table III).

[3]Ace Glass, Vineland, New Jersey, U.S.A.

Statistical analysis failed to reveal any significant differences in comparisons of lobar spore counts.

Animals 5N and 6N eliminated approximately 0.5 ml of the inoculum on exhalation following intranasal instillation. Animal 7N sneezed upon injection of the second nostril. On post-mortem examination of this animal regurgitated green feed constituents were seen in its trachea and major bronchi and spore counts obtained from these sites were rendered doubtful by this accident.

The numbers of spores inhaled by the four aerosol exposed calves 1H, 2H, 3L and 4L were calculated to be 9.4, 12.7, 14.4 and 19.3 x 10^7 spores respectively. However, the number of spores actually retained by these calves could not be calculated.

DISCUSSION

In calves exposed to the aerosol, spore counts were high in lung and trachea and low in the nasal cavity while the reverse occurred in the calves injected intranasally. This is not surprising as the Collison atomizer produces particles for the conditions of these tests (agent, diluent, concentration sprayed and relative humidity) which penetrate lung tissue beyond the smallest bronchioles. In man deposition of aerosols larger than 4 μ is decreased in alveoli but increased at head and tracheobronchial locations (22). The spores in lungs of animals exposed intranasally may have originated directly from the forceful injection and/or from the nasal cavities from which they became detached by the respiratory excursions and resuspended in the tidal air (12). The same mechanisms may have resulted

TABLE I. Details of Respiratory Challenge of Calves With Aerosols of Bg Spores

Calf #	Body Weight (kg)	Respirations during Aerosol Exposure	Total Tidal Volume (liter) (15)	Spores Inhaled/ Kg Body Weight	Temperature[a] °C	R.H.[b] %
1H	150	80	117	6.24 x 10^5	10	60
2H	173	100	159	7.35 x 10^5	10	60
3L	200	120	229	7.21 x 10^5	14	39
4L	318	100	304	6.05 x 10^5	14	39

[a]Temperature of exposure room
[b]Relative humidity of exposure room

in the correspondingly fewer spores in tracheae of lungs 5N and 6N. The high number of spores in the trachea of calf 7N is probably related to the washing effect of regurgitated and inhaled feed at the time of killing and post-mortem examination.

The actual number of spores deposited on respiratory tissue for the moment of last respiration of the calves is not known. The method of counting spores per gm of lung tissue included those spores which were deposited on mucosa and those in aerial

TABLE II. Spore Counts of Lung Tissues Following Aerosol Inhalation or Intranasal Instillation of Seven Calves

Lung Specimen[a]	Calf 1H x 10^4	Calf 2H x 10^4	Calf 3L x 10^4	Calf 4L x 10^4	Calf 5N	Calf 6N	Calf 7N
1	1.64	2.87	3.01	1.81	80	376	1596
2	2.19	5.47	2.90	2.34	80	52	1404
3	4.40	5.16	2.13	2.10	28	12	2720
4	2.55	4.58	3.36	2.05	32	56	1360
5	1.22	5.63	3.17	1.96	132	32	732
Mean	2.40	4.74	2.91	2.05	70	105	1562
6	2.06	3.91	2.10	4.64	568	28	316
7	2.46	4.66	3.77	2.70	240	36	120
8	3.89	6.55	2.88	3.81	1164	12	1304
9	2.32	6.98	2.90	2.22	228	52	2020
10	1.98	5.39	2.08	2.24	112	28	13080
Mean	2.54	5.49	2.75	3.12	462	31	3368
11	1.88	5.76	2.34	0.70	2800	224	80
12	5.39	3.21	1.70	2.14	7680	36	72
13	6.08	5.02	2.88	1.88	952	12	80
14	4.46	5.80	3.09	1.89	132	52	144
15	3.19	5.26	2.18	1.55	120	12	184
Mean	4.20	5.01	2.44	1.63	2336	64	112
16	1.38	3.10	0.59	0.86	152	64	980
17	4.06	4.64	1.34	1.14	144	84	1432
18	3.87	5.06	1.33	1.39	144	24	436
19	3.72	3.88	2.00	1.71	72	28	160
20	2.34	3.84	1.20	1.41	88	0	276
Mean	3.07	4.10	1.29	1.30	120	50	569
Mean	3.05	4.84	2.35	2.02	747	62	1424

[a]1-5 anterior lobe; 6-10 cardiac lobe; 11-15 anterior segments and 16-20 posterior segments of diaphragmatic lobe

TABLE III. Spore Counts of Pieces of Mucosa Taken from Areas in the Nasal Cavity and Trachea

Source of Specimens (2.5 cm^2)	Calf 3L	Calf 4L	Calf 5N	Calf 6N	Calf 7N[a]
Ventral turbinate (anterior) ...	896	8	3.76 x 10^6	9.40 x 10^5	2.60 x 10^5
Ventral turbinate (posterior)....	144	104	8.60 x 10^5	1.25 x 10^5	2.68 x 10^5
Posterior floor of nasal cavity...	2040	4080	1.25 x 10^6	3.52 x 10^5	not examined
5 cm distal from larynx.....	4560	5120	24	8	6446
5 cm proximal to apical bronchus	26720	2720	44	8	3920
5 cm distal to first bronchial bifurcation.....	15440	1784	16	128	12080

[a]Animal with regurgitated food

suspension at that site at the time of the last breath. Continuous respiration would have removed spores in aerial suspension from the tissue sites analysed.

The statistical analysis of lobar spore counts indicates that inhaled spores have equal opportunity to be deposited in any pulmonary segment. However, Lillie and Thomson (21) exposed calves to aerosol of *Pasteurella haemolytica* for 30 minutes and revealed less deposition in the posterior tips of diaphragmatic lobes than for the remainder of the lung. Regional differences of deposition of inhaled aerosols in the pulmonary lobes is of interest in relation to pneumonia of pigs and calves. In these species pneumonias are common and predominate in anterior lobes with progression to posterior segments. Results of this study indicate that inhaled insults are not concentrated in the anterior segments of the lung.

Direct application of antigen to respiratory tissue has been shown to produce significantly higher levels of local tissue immunoglobulin than parenteral application of the same antigen (11, 14). Consequently deposition of antigen on all critical sites of the respiratory tract is desirable. The present investigations indicate that deposition of spores takes place in all parts of the respiratory tract when the same inoculum is administered by intranasal installation or by aerosol inhalation. However, the amounts recoverable from various parts differ considerably. This may well be of importance in successful vaccination procedures or experimental infections of the respiratory tract, aerosol inhalation being the method of choice in exposure of trachea and lungs to vaccines or experimental infections.

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Book Review

TEXTBOOK OF THE ANATOMY OF THE DOMESTIC ANIMALS. *Edited by R. Nickel, A. Schummer and E. Seiferle. Vol. 5.* THE ANATOMY OF THE DOMESTIC FOWLS (ANATOMIE DER HAUSVÖGEL). *Edited by A. Schummer. Published by Paul Parey Verlag, Berlin and Hamburg. 1973. 203 pages. Price (in German) DM 96,-.*

The continuous expansion of the poultry industry as a pertinent part of today's animal science and husbandry encouraged the author to devote one of five volumes of this textbook series entirely to the anatomy of the domestic fowls including the chicken, turkey, guinea hen, goose, duck and pigeon. The anatomy of the chicken has received more emphasis over that of the other species due to the greater economic significance. Yet, morphological peculiarities occurring in the other species are not neglected. As in all volumes of this series, excellent illustrations accompany the text. Seven colored plates show very clearly the chicken's airsac system, types of blood cells, the circulatory system, as well as the topography of blood vessels and nerves of the various areas.

The introduction deals with the zoological classification and the descendance of the domestic fowls. The first chapter presents the skeletal and muscular systems and some 32 illustrations support the text. The Latin names of the muscles are listed in bold type for quick retrieval giving their origin, insertion, action and innervation.

The second chapter provides 104 pages of text and 83 illustrations covering the body cavities, digestive, respiratory, urogenical, circulatory systems, endocrine organs and nervous system. An adequate description of the spermiogenesis, oogenesis, development of the blood cells, formation of the blood, as well as the development and function of the egg membranes, has been incorporated into this chapter. The third chapter is devoted to sense organs with 12 pages and 12 illustrations. Eight pages with 14 illustrations on the integument followed by an extensive list of references to the international literature arranged according to organ systems and a comprehensive index terminate the textbook.

Undoubtedly, this textbook fills an essential gap in the veterinary literature and is an indispensable source of information for everyone concerned with avian anatomy. Printing, layout, paper and binding are impeccable and the illustrations are outstanding without exception. A translation of the German text would be desirable.

— *G. Speckmann.*