BACTERIAL FLORA OF BOVINE RESPIRATORY TRACT

by

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TABLE OF CONTENTS

INTRODUCTION

Bovine respiratory diseases are a major source of economic loss to the cattle industry. It was reported that 40-80% of all cattle diseases involve the respiratory system. Lillie (1974) conservatively estimated that losses to the Canadian cattle industry were millions of dollars annually.

It is currently accepted that a combination of factors are involved in the etiology of the bovine respiratory disease complex (Hamdy and Trapp, 1967). Recognized factors are stress induced by viral infection or environmental factors, in combination with bacterial colonization of the lower respiratory tract (Jericho and Langford, 1978).

Results of examination of lungs of cattle dead of the respiratory disease complex suggest involvement of Pasteurella sp. (Collier, 1968, and Jensen et al., 1976). However, attempts to reproduce the disease with cultures of Pasteurella sp. in animals not stressed or virus infected have been unsuccessful. It is, therefore, difficult to assess the pathogenic role of this group of bacteria.

Pasteurella spp. are recognized as part of the normal flora of the nasopharynx and trachea of cattle (Hamdy and Trapp, 1967; Saunders and Berman, 1964; Corstvet, 1973; and Frank and Wessman, 1978). They have not been recognized as a part of the normal flora of the bovine lung, but the composition of bacterial flora of the normal bovine lung has not been extensively studied. Collier and Rossow (1964) examined tissue from the diaphragmatic lobe of 88 cattle at slaughter and only recovered bacteria which were considered as inhaled soil inhabitants. All microorganisms recovered were considered as transient which were removed by the normal pulmonary defense mechanisms.

The purpose of this study was to examine the bacterial flora of the normal bovine lung and to determine if recovered bacteria represented transient or resident flora.

REVIEW OF THE LITERATURE

The role of bacteria in the pathogenesis of the bovine respiratory disease complex was not well understood. Most investigators suggested a complex etiology involving a combination of bacterial, viral and environmental stress factors. (Horlein et al., 1961; Collier et al., 1962; Hamdy and Trapp, 1967; Collier, 1968; and Gourley et al., 1970). Most surveys have incriminated Pasteurella spp. as the most predominant bacterial isolate from pneumonic lungs (Jensen et al., 1976), although a variety of other infectious agents including para-influenza-3 (PI-3) virus, Chlamydia sp., adenovirus, mycoplasmas, and infectious bovine rhinotracheitis (IBR) virus were recovered (Collier et al., 1962; Horlein et al., 1961; and Saunders et al., 1964). These species were also recovered with regularity from the nasal secretions of diseased and healthy feedlot cattle (Horlein et al., 1961; Collier et al., 1962; Hamdy and Trapp, 1967; Collier, 1968; and Gourley et al., 1970). The trachea also appeared to be a residual site for these microorganisms. Corstvet (1973) recovered Haemophilus somnus, Pasteurella sp., and Mycoplasma sp. from the trachea of healthy and diseased animals. He considered these as a part of the transient if not indigenous flora of the respiratory tract.

The presence of Pasteurella sp., or other infectious agents known to be associated with the bovine respiratory disease complex have not been demonstrated in apparently healthy lung tissue. Tracheal mucosa, lung hemogenates and bronchial lymphnodes of 88 apparently healthy cattle were examined by Collier and Rossow (1964). They recovered 510 isolates of bacteria and 8 isolates of common moulds prevalent in soil and feces. Bacillus sp. and Streptomyces sp. were most frequently recovered. They did not isolate pasteurellae and concluded that these were not associated with healthy tissues of the lower respiratory tract. None of the isolates appeared to be colonizing. They were considered transient flora which were recently inhaled. They

suggested that the lower respiratory tracts of cattle in dusty pens were subjected to a sustained shower of soil-borne microorganisms. Similarities were found between organisms isolated from the respiratory tract and those recovered from the bronchial lymphnodes. They concluded that the lymphatic system was important in clearance of microorganisms from the lungs.

An additional source of microorganisms in the lung was suggested by Mullenax (1964). He collected gas from the trachea of a cow and was able to recover microorganisms which normally inhabited the rumen. He concluded that bacteria may be eructated and inhaled.

Additional studies concerning the microflora of the lung of normal cattle were not found. However, a number of studies were conducted on the human with contradictory results. None or very few aerobic bacteria were found in most studies when specimens were obtained by bronchoscopy or transtracheal aspiration (Pecora and Yegian, 1958, and Nozzoli and Torelli, 1975). They concluded that normal human lung parenchyma was sterile. In a more recent study, Jordan et al., (1976) recovered six different genera of aerobes and seven different genera of anaerobes when specimens of tracheobronchial secretions were collected by fiber-optic bronchoscopy. Lindsay and Pierce (1978) examined the hypothesis that normal lung was sterile. They utilized the dog as a model and recovered aerobic bacteria from 37% of 268 lung samples from 19 dogs. They postulated that the lung was not a flawlessly sterile environment because bacteria from the pharynx were continuously aspirated, especially during sleep. Some of these bacteria were neither killed nor eliminated immediately by host defenses. They replicated in normal lung where they remained for varying intervals. It was not clear whether establishment of organisms in the lung was due to aspiration of unusually large numbers of organisms, a defect in the host defenses or both. They did not find evidence to support the theory that more bacteria occured in the ventral portion of the lung.

The pulmonary defense mechanisms were considered adequate to ensure sterility of the normal lung (Kaltreider, 1976). It was only when these mechanisms were impaired that microorganisms colonized and proliferated (Green, 1968).

It was postulated that the dynamics of deposition of inhaled particles in the respiratory tract obeyed the physical laws of inertia (Gareth and Green, 1967). This implied that the smaller the particle, the more distal it was deposited. It was calculated that 90% of inhaled particles with a diameter greater than 3µm were deposited on the mucosa from the distal bronchiole to the nasopharynx while 90% of those between 0.5 to 3µm were deposited in the alveoli and respiratory bronchioles. Particles of less than 0.5µm were usually not deposited and remained suspended in exhaled air (Kaltreider, 1976).

Jericho and O'Connel (1974) studied the deposition of Bacillus subitlis var. niger spores in the respiratory tract of cattle following inhalation and nasal instillation. Inhaled aerosolyzed spores were deposited more in the posterior segments of the lungs than spores in liquid suspension which were instilled intranasally. Statistical analysis of his results indicated that inhaled aerosolyzed spores were equally deposited in any segment of the tract. This seemed to contradict the findings of Lillie and Thompson (1972) who exposed calves to aerosols of P. hemolytica. They found fewer microorganisms in the posterior portions of the diaphragmatic lobe than in other parts of the lung.

The mechanism of pulmonary clearance of inhaled particles has been thoroughly studied. Appreciation of this mechanism would be relevant to understanding the respiratory tract environment. A filtering mechanism which served to trap large particulate matter suspended in inhaled air was present in the nasopharynx of mammals (Sisson and Grossman (ed) 1960). One to seven per cent of aerosolyzed P. multocida were recovered from

bovine lung tissue homogenates when administered by inhalation whereas 40-80% were recovered after intra-bronchial injection (Flossman, 1977).

The respiratory tract mucosa was coated by a mucus secretion that was of a special physical consistency. It contained proteolytic enzymes and offered physical, chemical, and immunologic barriers to invading microorganisms (Kaltreider, 1976). It flowed anteriorly, moved by the biphasic whiplike motion of the cilia of the epithelial cells that lined the respiratory tract from the distal bronchiole to the nasopharynx (Green, 1968). It was described as an escalator because it carried deposited particles from the distal bronchioles to the nasopharynx (Green, 1968). The rate of movement in man was measured at 10-20 mm/min., culminating in clearance of more than 90% of total deposited material in less than 60 minutes (Kaltreider, 1976).

Lillie and Thompson (1972) compared the rate of clearance of bacteria from the lungs of white mice and calves. They found that calves cleared these agents more rapidly than mice. The difference was not attributed to mucociliary activities. Pulmonary macrophages were concluded to be more active in bovine than in murine lungs.

Non ciliated epithelial cells lined the mucosal surfaces of the respiratory bronchioles and alveoli of mammals (Kaltreider, 1976). Inhaled particles deposited in these regions were removed by more complex systems. The rate of fluid flow in these regions was very slow and rated in days and years (Kaltreider, 1976). The mechanisms of flow were poorly understood. Alveolar macrophages played a dominant role in removal of particulate matter from these regions. Those which were ladden with engulfed particles migrated to the distal bronchioles from where they were carried to the nasopharynx via the mucociliary escalatory mechanism. Those particles not engulfed by alveolar macrophages were drawn into the lymphatic drainage system at special areas on the mucosa described as "lympho-

epithelial organs". These blind pocket origins of lymphatic ducts exerted negative pressure on the content of the alveoli and respiratory bronchioles (Kaltreider, 1976). Such particles, if they persisted and were not degraded, usually ended up in regional lymph nodes. Particles not removed by either of the above mechanisms penetrated the respiratory epithelium and entered the interepithelial connective tissue where they were engulfed by histiocytes (Kaltreider, 1976).

Living particulate matter such as bacteria and viruses, were rapidly neutralized in a specific manner by the immune defense mechanism of the respiratory system. This system has been extensively studied. Immunoglobulins of the lgA, lgG, lgM and lgE classes were reported present in respiratory tract secretions of the dog (Kaltreider, 1976). These antibodies occured in a relatively higher concentration in pulmonary secretions than could be explained by transudation from intravascular fluid. Immunofluorescence studies of submucosal lymphoid tissue indicated local production (Martinez - Tello et al., 1968).

Immunoglobulin G was most effective in combating bacterial invasion of the lower respiratory tract. It fixed complement which was demonstrated to be present in low levels in normal bronchial secretion but increased with inflammation (Johnson and Philip, 1977). Alveolar macrophages had receptor sites for the Fc portion of IgG molecule which facilitated bacterial opsonization (Fundenberg et al., (ed) 1976).

Immunoglobulin A was most effective as a neutralizing antibody but less effective in combating bacteria. It blocked receptor sites on invading microorganisms, thereby preventing them from attaching to mucosal surfaces. It neutralized inhaled toxic macromolecules and exerted an antibacterial effect in conjunction with lysozyme or lactoferrin (De Coteau, 1974). Cell mediated immunity was involved in the defense mechanism of the lung. Locally produced T-cells elaborated lymphokines that affected alveolar

macrophages (Johnson and Philp, 1977). The latter became activated and more competent in their ability to destroy bacteria.

Gerbrandy and Dura (1972) demonstrated an anamnestic response involving immunoglobulins in the respiratory tract. Gadol and Johnson (1974) concluded that pulmonary T-lymphocytes exhibited memory, but pulmonary B-lymphocytes did not.

Certain agents were known to impair pulmonary defense mechanisms. Para-influenza-3 (PI-3) virus destroyed cilia lining the upper respiratory tract. It was also shown to impair ingestion and killing of bacteria by mouse alveolar macrophages (Warshaur, 1977). In the latter case, the reaction was optimum when mice were challenged with bacteria 7 to 11 days post exposure. There was no noticeable impairment of macrophage activity when mice were challenged on post-exposure day three.

Inert dust did not enhance bacterial colonization in hamsters, although, it seemed to favor infection of the lungs by mycoplasma (Battigel, 1971).

Ozone, as a pollutant, decreased pulmonary bactericidal effects (Goldstein et al., 1971). In humans tobacco smoke caused production of large amounts of activated macrophages (Johnson and Philp, 1977). Their lysosomal enzymes caused damage to pulmonary tissues when released. Pulmonary edema retarded alveolar macrophage activities (Marc-Laforce, 1973).

The pathogenesis of shipping fever pneumonia in cattle has not been well understood. In humans, presence of low levels of bacteria in the lungs was reported as a possible cause of emphysema (Lindsay and Pierce, 1978). Some bacteria were reported to have alpha-antitrypsin inhibitory capacity in vitro. Others produced a mild secondary inflammatory response which incited release of macrophage or leukocyte protease (Lindsay and Pierce, 1978). Tissue destruction resulting from subsequent enzymatic degradation of lung tissue might lead to the development of emphysema.

Jensen (1976) hypothesized that endotoxin from Pasteurella sp. formed thrombi which occluded lymphatics, capillaries and veins in infected lobules resulting in ischemic neorosis.

MATERIALS AND METHODS

Collection of Specimens

Fluids were collected on sterile cotton tipped applicators from the tracheal and bronchial mucosa of 50 bovine lungs at slaughter.* The beef cattle from which specimens were collected were of assorted sex and breed. The animals were estimated at 18-24 months of age and graded good to prime. Animals were slaughtered at a rate of approximately 300 per hour, and continually arrived at the plant by truck. No attempt was made to select animals from a particular area and they originated from several feedlots in Kansas and Nebraska (figure 2).

Two or four lungs were collected and examined at one or two week intervals from July to November, 1978. Specimens were collected only from lungs which were free of gross lesions and animals on which edible parts were passed for human consumption.** Spillage of gastro intestinal content onto any part of the viscera was selected as a criterion for rejection of the lung. Estimated time from stunning to evisceration was 30 minutes. Immediately after evisceration, the selected lungs were removed from the line, taken to a clean area and placed in a sanitized plastic container. The trachea and bronchi were opened with sanitized scissors which were placed in 95% ethanol and flamed before cutting into each area of the lung sampled. Fluids were collected from the mucosa of ten portions of the tracheo-bronchial tree (figure 1). Areas sampled were:

(a) The trachea at a level 12-13 cm cranial to its bifurcation

(b) The tracheal bifurcation

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*Iowa Beef Processors, Inc., Emporia, Kansas.
**United States Department of Agriculture, Meat Inspection Division

(c) The distal bronchi at a level just large enough for passage of the applicator (approximately 4 mm diameter) in the following regions:

(1) Right cranial apical lobe

(2) Right caudal apical lobe

(3) Cardiac lobe

(4) Right diaphragmatic lobe

(5) Accessory lobe

(6) Left cranial apical lobe

(7) Left caudal apical lobe

(8) Left diaphragmatic lobe

Following collection, swabs were immediately placed into 1 ml of sterile phosphate buffered saline (PBS) in screw capped tubes. The portion of the swab in contact with the hand was broken off and discarded. Collection of specimens from four lungs required approximately 90 minutes.

Inoculation of Bacteriologic Media

Within 20 minutes of collection of the last specimen, they were taken to a local laboratory* for culturing. Each tube was agitated on a vortex mixer for 30 seconds to suspend fluids and bacteria in the PBS. The swab was pressed against the side of the tube to express excess PBS, aseptically removed and transferred to 4 ml of Tryptic soy broth**. Four drops of the PBS were then placed on the surface of four or five different culture media in plastic disposable petri dishes,*** and streaked for isolation.

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*Department of Bacteriology, Emporia State University, Emporia, Kansas.
**Difco Laboratories, Detroit, Michigan.
***Fisher Scientific Co., St. Louis, Missouri.

The primary plating media utilized were:

(1) Blood Agar (BA) - Trypticase soy agar* plus 5% citrated bovine blood.

(2) MacConkey Agar** (MAC)

(3) Phenylethyl Alcohol Agar (PEA)* plus 5% citrated bovine blood

(4) Chocolate Agar (CA) - Trypticase soy agar* plus 1% Hemoglobin** and 1% IsoVitalex**

OR

Lysed Blood Agar (LBA) - Trypticase soy Agar* plus 10% citrated bovine blood which had been frozen and 0.25 gm per litre BETA DPN***.

(5) Thayer-Martin Agar (TM) - Mueller-Hinton Agar** plus 1% Hemoglobin**, 1% IsoVitalex** and 1% V-C-N Inhibitor* (Vancomycin 300 mg. Colistin 750 mcg and Nystatin 1,250 units per ml).

Following sampling of the tenth lung, the supply of IsoVitalex** was exhausted. Attempts to replenish the supply were unsuccessful at that time because all local suppliers were unable to obtain the product from the manufacturer**. At that point, lysed blood agar was substituted for chocolate agar and Thayer-Martin agar was dropped as a primary plating medium. All batches of both chocolate agar and lysed blood agar were tested for their ability to support the growth of an "X" and "V" factor requiring Haemophilus sp. and Haemophilus somnus throughout the project. The petri dishes were packed into polyethylene bags and stacked horizontally in an empty ice chest for the trip back to Manhattan, Kansas.

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*Baltimore Biological Company, Baltimore, Maryland.
**Difco Laboratories, Detroit, Michigan.
***Sigma Chemical Company, Baltimore, Maryland.

Within two hours of the time of plating the last specimen, the plates and tubes of TSB were placed in a 37C aerobic incubator with a 5% increased Co_2 tension. Tubes of TSB were incubated for 24 hours and one loopful streaked for isolation on Mannitol Salt Agar* plates and incubated at 37C.

Identification of Isolates

Following 18, 48 and 72 hours incubation, plates were examined and each colony type enumerated, described and subcultured. Each different type of microorganism recovered was preserved by freezing on glass beads at -60C (Nagel and Kunz, 1972). Pure cultures of each microorganism were identified when possible, using generally accepted procedures and keys (Buchanan and Gibbons (ed.) 1974; Gordon et al., (1973); Kloos and Schleifer, 1974; Kloos et al., 1975; Lennet et al., (ed.) 1974; Smith and Bettge (1972); Schleifer and Kloos, 1975; Weaver et al., (1974).

*Difco Laboratories, Detroit, Michigan.

Statistical Analysis

The enumerated colony forming units from one petri dish of a particular medium on which the microorganism grew, was most numerous and well isolated, were utilized for statistical analysis.

These numbers were analyzed in an attempt to determine whether microorganisms recovered from various locations in the lung were inhaled and transient residents or were actively colonizing and proliferating in the fluids. This analysis was based on the assumption that a definite number of microorganisms of a particular type should be recovered from a particular location to be considered as colonizing. It was assumed that in the absence of colonization, the distribution of an organism in respect to numbers recovered would be random and that this rendomness would fit a generalized Poisson probability model (Cohen, 1960). The procedure adopted for fitting a Poisson probability model to the data was sequential. The model was first fitted to all data collected on each genus of bacteria collected at a sampling site. A chi square goodness of fit (Snedecor and Cochran (ed) 1967) was used to test the adequacy of the model. When inadequate, the data was truncated by removal of the most extreme class. Truncation continued until an ordered subset of classes starting at X=0 to X= an indefinite number (K) was found which adequately fit the Poisson model. Where X stands for the number of colonies of a particular genus isolated from a particular sampling site. The probability of making each observation of X under the adopted model was calculated. For example of the method, see appendix (table 51).

To determine if any particular area of the tract was more predominantly colonized, a chi square goodness of fit for uniform distribution was utilized (Snedecor and Cochran (Ed.) 1967).

FIGURE I

Diagrammatic representation of the bovine respiratory tract (Dorsal view). Locations from which secretions were collected are designated A-J.

A - Trachea

B - Tracheal bifurcation

C - Right cranial apical

D - Right caudal apical

E - Cardiac

F - Right diaphragmatic

G - Accessory

H - Left cranial apical

I - Left caudal apical

J - Left diaphragmatic

Figure 1

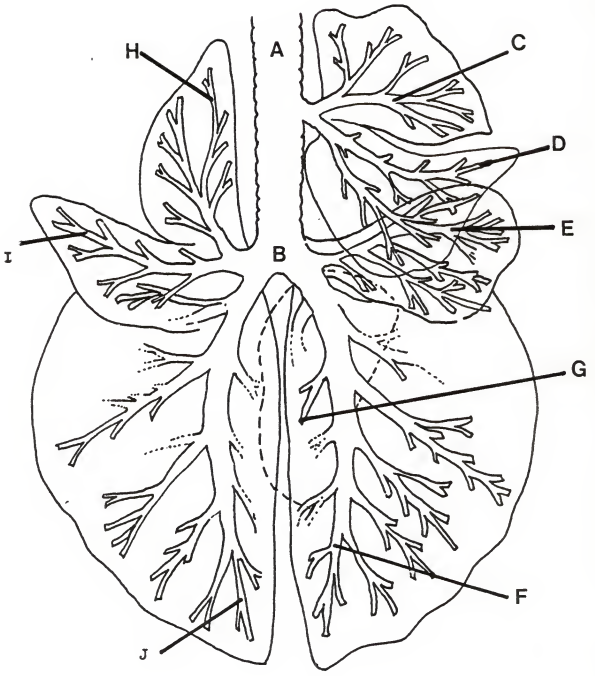
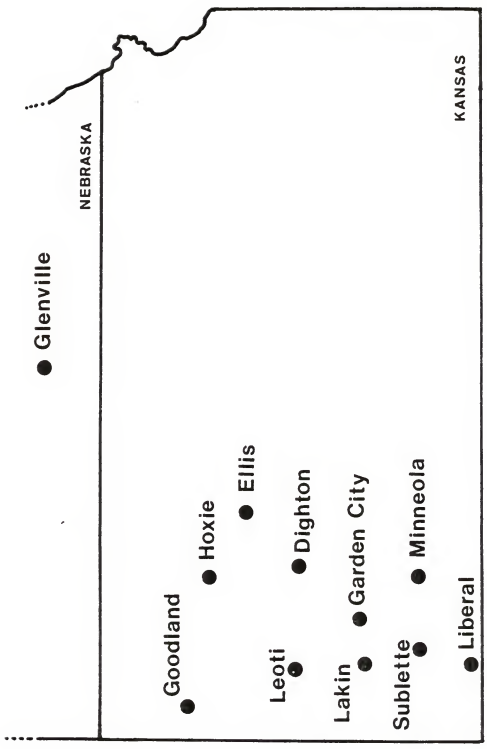


FIGURE 2

Distribution of feedlots on which examined cattle were raised.

Figure 2


Glenville
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Goodland
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Leoti
Dighton
Lakin
Garden City
Sublette
Minneola
Liberal

RESULTS

Aerobic bacteriological examination of tracheal and bronchial fluids from 50 bovine lungs resulted in recovery of bacteria belonging to 20 genera. Additional isolates were placed into two Centre for Disease Control (CDC) alpha-numeric designations. Microorganism recovery frequencies by location sampled are presented in Table 1.

A total of 433 isolates was recovered from 48 of the respiratory tracts. Two of the tracts failed to yield growth of any bacteria from any of the locations sampled. More isolates were recovered from the trachea than any other single location. They accounted for 112 (25.9%) of the total isolates. The second most frequent source of isolates was the tracheal bifurcation 80 (18.5%). Other locations sampled yielded from 22 (5.1%) to 38 (8.8%) of the isolates (Table 4).

Based on the statistical analysis, a total of 146 of the recovered isolates were considered as colonizing the tracts (Table 2). The number of organisms isolated from a given location which were considered as colonizing varied markedly from one bacterial genus to another and from one sampling site to another. This number varied from as few as three colony forming units (CFU) to as many as too numerous to count.

Eight of the tracts did not yield enough CFU's of any one microorganism in any single location to be considered colonized. Since there was no growth from two tracts, forty tracts were considered colonized. Twenty of the tracts were colonized by only 1 genus, 5 by 2, 10 by 3, 3 by 4, 1 by 5 and 1 by 9.

Seventeen genera and one CDC alpha-numeric designation recovered were considered colonizing. The frequency of colonization by location is presented in Table 2. Members of the genus Streptomyces were found most frequently

as a colonizer (29.5%). Pasteurella sp. represented 13% of the colonizing isolates. These species colonized a total of 19 locations in 9 tracts. The sites most frequently colonized by Pasteurella sp. were the trachea and its bifurcation, but they were recovered in at least one instance from all but two (left cranial apical and left diaphragmatic) sites. Pasteurella sp. were the second most widely distributed colonizing microorganism recovered. They were recovered from an additional 9 tracts, but not in sufficient numbers to be considered colonizing.

The frequency of colonization of the various sampling sites without regard to genus is presented in Table 3. The trachea and tracheal bifurcation were the areas most frequently colonized. They were colonized more frequently than other locations at a probability (P) less than 0.001 by the chi square test for goodness of fit for uniform distribution. They were not significantly different from each other. The frequency of colonization of other sampling sites ranged from 4 to 11 of the 50 lungs sampled. There was no significant difference (P=.63) between the degree of colonization in these sites.

CDC Alpha-numeric Designations: Organisms in this class were recovered at low frequencies. One lung was colonized in the right caudal apical lobe by IIc. Groups IIf, IIb and IVf were recovered from a total of 4 tracts and were considered transient flora.

Gram Negative Aerobic Rods: A single colony of Bordetella bronchiseptica was recovered from the accessory lobe. Six species of pseudomonads were recovered from a total of 9 lungs. One lung was colonized in the left diaphragmatic lobe by Pseudomonas acidovorans. The pseudomonads comprised 2.3% of total isolates.

Gram Negative Cocci and Coccobacilli: Members of the genus Neisseria represented 7.3% of total isolates. Twenty-eight isolates were identifiable only as Neisseria sp. One particular isolate was very dysgonic. It was

wet, flat and irregular. At 18 hours incubation, colonies were 5mm in diameter with a narrow zone of complete hemolysis. They were found colonizing the trachea and tracheal bifurcation. They did not survive preservation and were not further characterized. Neisseria mucosa was recovered from the left apical lobe, and Neisseria sicca colonized the right diaphragmatic.

Branhamella catarrhalis was recovered from 2 lungs. It was found along with P. hemolytica colonizing the trachea, tracheal bifurcation and accessory lobe of one lung.

Eight isolates of moraxellae were identified to the species level. Four isolates were referred to as Moraxella sp. because they could not be speciated using available keys. Seven tracts were colonized in 8 locations by members of the genus Moraxella.

Gram Negative Facultatively Anaerobic Rods: Three recognizable species of pasteurellae were recovered. Pasteurella hemolytica represented 61.8% and P. Multocida 32.4% of total pasteurellae isolates. Pasteurella gallinarium was recovered once from the trachea. One isolate was identified as Pasteurella sp. It was a Gram-negative pleomorphic bacillus. It produced acid over acid on triple sugar iron agar* (TSI), a positive oxidase reaction and reduced nitrate. It was indole, urea and citrate negative. Acid was produced in 1% glucose and maltose in heart infusion broth*. It did not produce acid in 1% xylose, mannitol, lactose or sucrose.

Enterobacteria were recovered at low frequencies. Enterobacter liquefaciens colonized two lungs of cattle from the same feedyard. Escherichia coli was isolated from three lungs, but colonized the trachea of only one.

Three isolates of a Haemophilus-like organism colonized the trachea and tracheal bifurcation of one lung and the tracheal bifurcation and left

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*DIFCO Laboratories, Detroit, Michigan

diaphragmatic lobe of another. These organisms were very dysgonic and were gram-negative small rods. A twenty-four hour growth on blood agar produced colonies that were round, greyish, glistening, smooth, slightly raised and 1-2 mm in diameter. A complete zone of hemolysis surrounded each colony. They were oxidase positive. The cultures did not grow on any differential media without the addition of serum. They produced indole and produced acid from maltose, xylose, lactose, sucrose, mannitol and glucose.

Aeromonas hydrophila colonized the diaphragmatic lobe of one lung.

Gram Positive Cocci: Ten per cent of the isolates were staphylococci. Goagulase positive staphylocci were not recovered. All recovered staphylococci were enumerated on any of three primary plating media, namely BA, PEA and, LBA. There was no occasion when staphylococci were isolated on mannitol salt agar without being isolated at the same time on any of the previously mentioned media. All 47 isolates were identified as to species. Staphylococcus epidermidis was the most predominant. Five other species were isolated with less frequency.

Micrococcus sp. comprised 6% of all isolates. They were recovered from 20 lungs and were separated from the staphylococci by their inability to produce acid aerobically from 10% glycerol in purple agar base plus 4mg/ litre of streptomycin (Schleifer and Kloos).

Five serological groups of streptococci were recovered. These comprised 9% of total isolates. They were classified according to the Lancefield scheme. Group D was the most predominant. A single colony of Group A streptococcus was recovered from the trachea of one animal.

Endospore forming Rods: Eight species of Bacillus were identified. Bacillus pumilus and B. subtillis predominated and were recovered from 12 and 11 lungs respectively. Members of the genus Bacillus comprised 10% of total isolates, but colonized only 3 lungs.

Gram Positive Asporogenous Rod-shaped Bacteria: Lactobacilli were recovered from the tracheal bifurcation on two occasions. However, conditions of this study were not optimal for recovery of these organisms.

Actinomycetes and Related Organisms: This was the most predominant class of organism recovered. Corynebacterium sp. comprised 11% of all isolates, and were recovered from 31 lungs, and colonized 10. No recognizable species was identified and were best classified as diphtheroids.

Streptomyces sp. were the most numerous genus and represented 23% of all isolates. They were recovered from 30 lungs and colonized 15. Two of the lungs were colonized in all examined locations. No attempt was made to speciate members of this genus.

Table 1. Isolation frequency of Bacteria from the respiratory tracts of 50 cattle.

Microorganisms	Number of Cattle Harboring By location										Total
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]	10[j]	
CDC Alphanumeric Designations											
Group IIc	-	-	1	-	-	-	-	-	-	-	1
Group IIF	-	-	1	-	-	1	-	1	-	-	2
Group IIB	-	-	-	-	-	-	-	-	-	1	1
Group IVF	-	-	1	-	-	-	-	-	-	-	1
Gram-negative Aerobic Rods											
Pseudomonas putida	-	1	-	-	-	-	-	-	-	-	1
Pseudomonas testosteroni	1	-	1	-	-	-	-	-	-	-	2
Pseudomonas auruginosa	1	-	-	1	-	-	1	-	-	-	2
Pseudomonas maltophilia	-	-	1	-	-	-	-	1	-	-	2
Pseudomonas acidovorans	-	-	-	-	-	-	-	-	-	1	1
Pseudomonas diminuta	1	-	-	-	-	-	-	-	-	-	1
Bordetella bronchiseptica	-	-	-	-	-	-	1	-	-	-	1

Table 1. (continued)

Microorganisms	Number of Cattle Harboring By location										Total
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]	10[j]	
Gram-negative cocci and coccobaccilli											
Neisseria mucosa	-	-	-	-	-	-	-	-	1	-	1
Neisseria sicca	1	-	-	-	-	1	-	-	-	1	2
Neisseria sp.	5	7	1	2	1	2	1	4	2	3	16
Branhamella catarrhalis	2	1	-	-	-	-	1	-	-	-	2
Moraxella osloensis	4	2	-	-	-	-	-	-	-	-	4
Moraxella Liquefaciens	1	1	-	-	-	-	-	-	-	-	1
Moraxella sp.	1	2	1	-	-	-	-	-	-	-	3
Acinetobacter calcoaceticus var. anitratus	2	-	-	1	-	-	1	2	-	-	4
var. lwoffi	-	-	1	1	1	-	-	1	-	-	2
Gram-negative Facultatively anaerobic rods											
Pasteurella haemolytica	8	5	1	1	1	1	1	1	1	1	11
Pasteurella multocida	4	3	-	-	-	1	1	-	1	1	5
Pasteurella gallinarum	1	-	-	-	-	-	-	-	-	-	1

Table 1. (continued)

Microorganisms	Number of Cattle Harboring By location										Total
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]	10[j]	
Gram-negative Facultatively anaerobic rods											
Pasteurella sp.	-	-	-	-	-	1	-	-	-	-	1
Escherichia coli	1	1	-	-	-	-	-	1	-	-	3
Enterobacter liquefaciens	1	-	1	-	1	-	1	-	1	2	3
Aeromonas hydrophila	-	-	-	-	-	-	-	-	-	1	1
Citrobacter freundii	1	-	-	-	-	-	-	-	-	-	1
Flavobacterium sp.	1	-	-	-	-	-	-	-	-	-	1
Haemophilus-like	1	2	-	-	-	-	-	-	-	1	2
Gram-positive Cocci											
Micrococcus sp.	7	3	2	2	3	2	1	-	4	2	20
Staphylococcus simulans	2	1	-	-	-	-	1	-	-	-	3
Staphylococcus epidermidis	4	1	3	1	-	1	3	6	2	3	10
Staphylococcus xylosus	1	3	-	-	-	1	-	-	-	1	3
Staphylococcus hominis	3	2	1	-	-	-	-	-	1	-	3
Staphylococcus warneri	1	-	-	-	-	1	-	1	1	-	3

Table 1. (continued)

Microorganisms	Number of Cattle Harboring By location										Total
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]	10[j]	
Gram-positive Cocci											
Staphylococcus cohnii	1	1	-	-	-	-	-	-	-	-	1
Streptococcus sp. Group F	2	2	1	1	-	-	-	-	-	1	3
Streptococcus sp. Group D	3	6	-	-	2	1	3	2	1	1	11
Streptococcus sp. Group B	4	1	1	1	1	-	1	-	1	-	8
Streptococcus sp. Group A	1	-	-	-	-	-	-	-	-	-	1
Streptococcus sp. Group C	1	-	-	-	-	-	-	-	-	-	1
Endospore forming Rods											
Bacillus subtilis	5	4	1	2	-	3	-	-	2	-	11
Bacillus pumilus	3	3	-	4	1	2	2	-	-	2	12
Bacillus firmus	1	-	-	-	-	-	-	-	-	-	1
Bacillus sphericus	-	-	-	-	-	-	-	1	-	-	1
Bacillus laterosporus	1	-	-	-	-	-	-	-	-	1	2
Bacillus circulans	1	-	-	-	-	-	-	-	-	1	2

Table 1. (continued)

Microorganisms	Number of Cattle Harboring By location										Total
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]	10[j]	
Endospore forming Rods											
Bacillus stearothermophilus	-	1	-	2	-	1	-	-	-	-	2
Bacillus megatarium	-	-	1	-	-	-	-	-	-	-	1
Gram-positive asporogenous rod-shaped Bacteria											
Lactobacillus sp.	-	2	-	-	-	-	-	-	-	-	2
Actinomycetes and related Organisms											
Corynebacterium sp.	13	9	4	2	3	6	3	4	2	4	31
Streptomyces sp.	21	16	4	7	8	11	9	8	5	10	30

[a]Trachea
[b]Tracheal bifurcation
[c]Right cranial apical lobe
[d]Right caudal apical lobe
[e]Cardiac (Middle) lobe
[f]Right diaphragmatic lobe
[g]Accessory (Intermediate) lobe
[h]Left Cranial apical lobe
[i]Left caudal apical lobe
[j]Left diaphragmatic lobe

Table 2. Colonization frequency of respiratory tracts of 50 cattle.

Microorganisms (genera)	Number of Lungs Colonized by Location										Total
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]	10[j]	
Streptomyces Spp.	10	9	2	2	2	4	4	2	3	5	15
Bacillus Spp.	1	1	-	1	-	-	-	-	1	-	3
Corynebacterium Spp.	3	-	2	-	2	3	1	-	2	-	10
Staphylococcus Spp.	3	-	1	-	-	-	2	3	-	-	7
Micrococcus Spp.	4	2	-	-	-	-	-	-	1	1	5
Streptococcus Spp.	6	3	-	1	-	1	-	2	-	1	10
Moraxella Spp.	2	4	1	-	-	-	-	-	-	1	7
Neisseria Spp.	5	3	1	-	1	1	-	-	-	1	8
Acinetobacter Spp.	-	-	-	1	-	-	-	-	-	-	1
Branhamella Spp.	1	1	-	-	-	-	1	-	-	-	1
Pasteurella Spp.	7	5	1	1	1	1	2	-	1	-	9
Pseudomona Spp.	-	-	-	-	-	-	-	-	-	1	1

Continued

Table 2 Continued.

Microorganisms (genera)	Number of Lungs Colonized by Location										Total
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]	10[j]	
Escherichia Spp.	1	-	-	-	-	-	-	-	-	-	1
Enterobacter Spp.	-	-	-	-	-	-	1	-	-	2	3
Aeromonas Spp.	-	-	-	-	-	-	-	-	-	1	1
Haemophilus-Like Spp.	1	2	-	-	-	-	-	-	-	1	2
GROUP II (CDC Alpha-numeric designation)	-	-	1	-	-	-	-	-	-	-	1
Lactobacillus Spp.	-	1	-	-	-	-	-	-	-	-	1

a. Trachea

b. Tracheal bifurcation

c. Right cranial apical lobe

d. Right caudal apical lobe

e. Cardiac lobe

f. Right diaphragmatic lobe

g. Accessory lobe

h. Left cranial apical lobe

i. Left caudal apical lobe

j. Left diaphragmatic lobe

Table 3. Frequency of colonization of different locations in the respiratory tract of 50 cattle.

Location	Number of Lungs Colonized	Percentage
Trachea	25	50%
Tracheal bifurcation	24	48%
Right cranial apical lobe	4	8%
Right caudal apical lobe	6	12%
Cardiac lobe	6	12%
Right diaphragmatic lobe	9	18%
Accessory lobe	9	18%
Left cranial apical lobe	5	10%
Left caudal apical lobe	7	14%
Left diaphragmatic lobe	11	22%

Table 4. Isolation frequency of aerobic bacteria from various locations in the respiratory tracts of 50 cattle.

Location	Number of Isolates	Percent of Total Isolates
Trachea	112	25.9
Tracheal bifurcation	80	18.5
Right cranial apical lobe	28	6.5
Right caudal apical lobe	28	6.5
Cardiac lobe	22	5.1
Right diaphragmatic lobe	36	8.3
Accessory lobe	31	7.2
Left cranial apical lobe	33	7.6
Left caudal apical lobe	25	5.8
Left diaphragmatic lobe	38	8.8
Total	433	

DISCUSSION

Four hundred thirty-three isolates of bacteria, some of which were known pathogens were recovered from fifty bovine lungs in varying numbers and frequencies. Thus a hypothesis that the lung is sterile (Pecora and Yegan, 1958) cannot be advanced for feedlot cattle. The ecological status of recovered bacteria varied from transient to colonization. It was apparent that the pulmonary defense mechanism was not adequate to maintain sterility within the respiratory tract. The reasons for this would need to be further studied. A few possibilities will be discussed.

The concentration of microorganisms in inhaled air especially in dusty pens could overwhelm the mechanisms of pulmonary clearance. This conforms with the suggestion of Collier and Rossow, (1964), who examined the respiratory tracts of 88 healthy cattle at slaughter and recovered 510 isolates of bacteria prevalent in soil and feces.

Cattle raised under feedlot conditions may be defective in pulmonary defense mechanism. If the rate of clearance of particulate matter by the mucociliary escalatory mechanism in the bovine is comparable to that established for man, no bacteria would be able to stay long enough in the respiratory tract to proliferate and colonize. However, if there was a flaw in the mechanism, the clearance of inhaled particles would not be thorough. Such a flaw could be due to presence of areas on the respiratory tract mucosa lined by epithelial cells that lack cilia. Such places could serve as "islands" on which microorganisms might have settled and proliferated. The "lymphoepithelia organs" (Kaltreider, 1976) could serve as such a site. Similar organs in the gastro-intestinal tract of mouse (lymphoepithelium overlying Peyer's patches) have been recognized as sites for bacterial proliferation (Abrams, 1977). Viruses have been reported to destroy ciliary epithelium

lining the upper respiratory tract (Jericho and Langford, 1978).

Tracheo-bronchial secretions in feedlot cattle may be exceptionally rich in substances which could be utilized by some bacteria for growth. This speculation was raised because some organisms that were dysgonic on enriched laboratory media were recovered from the respiratory tract in numbers that indicated they were growing luxuriantly in fluids of the tract.

The immune systems associated with the respiratory tract of beef cattle may be defective. Some organisms that produce mucinase could penetrate the physical barriers offered by the mucus lining of the respiratory tract, attach to the surface of epithelial cells and proliferate. Vibrio cholera and Campylobacter fetus are known to have mucinase activities (Burnet, 1948; Dennis, 1967). The trachea and tracheal bifurcation which were more frequently colonized than the rest of the tract appeared to be the source of organisms that later colonized the lower regions. As there was no significant difference in levels of organisms recovered from the different lobes of the lung, a rational conclusion would be to postulate that organisms were carried to these regions suspended in inhaled air. Particles that are thus distributed have equal chances of being deposited within any lobe. If they were distributed as a suspension in aspirated fluid, they would tend to concentrate in a ventrally situated lobe such as the accessory lobe.

On a few occassions, organisms that were not recovered from the trachea were found colonizing the bronchi. An example was Aeromonas hydrophila which was recovered from the left diaphragmatic lobe in numbers that were too numerous to count and was not recovered from any other location. In such an instance, it was possible that the organism invaded the lung via a hematogenous route or it may have been present in inhaled air in such low numbers that only one organism was deposited at a site which it colonized. On the other hand, this isolate could have been deposited in the trachea,

but did not survive due to unfavorable conditions, one of which might be competition with numerous other organisms for growth factors.

An important result obtained from these studies was the recovery of Pasteurella spp. from 18 of the 50 lungs examined. Nine of the lungs were colonized. Collier and Rossow (1963) reported that Pasteurella spp. were not associated with apparently healthy lung tissue in the bovine. The results of this study contradicted the above. Pasteurella spp. were found colonizing at least once in eight of the ten locations examined. It is possible that the stress to which animals were subjected prior to slaughter may have contributed to colonization of the bronchi by pasteurellae. Cavallero and Sala (1951) described the effect of corticosterone on latent pasteurella infection in mice. This substance is released in the body as a result of physiological stress and has been implicated as a possible cause for enhancement of pasteurella infection.

It was reported that some strains of pasteurellae were virulent while others were not (Carter and Bain, 1960). The pasteurellae that were found in this survey were not serotyped. There were no gross pathologic lesions associated with the lungs which were colonized. The potential virulence of these isolates was not determined. However, virulence factors which have been extensively studied in some species of bacteria are not well known in pasteurellae. Plasmids are known to encode for the synthesis of various factors in Escherichia coli (Magdalene et al., 1978; Gyles et al., 1978; Bouanchaud et al., 1975; Orskov and Orskov, 1973). It is known that such plasmids are transferable during conjugation or by lysogenic bacteriophage. Takeda and Murphy (1978) demonstrated the conversion of a non-enterotoxigenic E. coli to an enterotoxigenic strain by the latter method. Bacteriophage have been associated with pasteurella (Gadberry and Miller, 1978). Similar studies, if applied to Pasteurella could yield valuable results.

Physiological stress was believed to increase susceptibility of animals to pasteurellae infection and heighten virulence of the organism (Carter and Bain, 1960). Cavalero and Sala (1951) demonstrated the effect of steroid hormones on the virulence of P. multocida in rats. Latent infections were made acute and lethal by inoculation of cortisone, a substance which is released in the body as a result of physiological stress. This suggests that physiological conditions may alter the characteristics of pasteurellae. A saprophytic phase could turn parasitic and virulent if provided with favorable conditions. It would thus be considered an opportunistic pathogen.

The mechanism by which Pasteurella spp. cause diseases is not well known. A toxic pyrogenic lipopolysaccharide was isolated from type B strain of P. multocida (Carter and Bain). Jensen et al., 1976, suggested that pasteurellae endotoxin formed thrombi which occluded lymphatics, cappillaries and veins resulting in ischemic necrosis of the infected tissues.

Hemophilus somnus was not recovered from the respiratory tract in this study. Corstvet et al., (1973) found H. somnus in the trachea of living feedlot cattle and concluded that they were part of the transient, if not indigenous, flora of the respiratory tract.

Two organisms were recovered which were classified as Haemophilius-like. They grew in heart infusion broth to which serum was added. It was possible to determine that they formed acid from maltose. Recent reports indicated the possibility of false positive maltose reactions in serum-supplemented media. This was attributed to the presence in serum of a substance which hydrolysed maltose to two molecules of glucose.*

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*Hollis D.G., Riley P.S., and R.E. Weaver. Center for Disease Control, Atlanta, Georgia. Serum supplementation as a cause of false positive maltose reactions: Amended Description of Kingella denitrificans. Abst., 79th Annual Meeting, American Society for Microbiology, Los Angeles, California, 1979.

Streptomyces were the most frequently recovered organisms. Their presence in the respiratory tract would suggest inhalation of soil-borne particles since this group of microorganisms is commonly found in the soil (Stanier et al.). Some species of streptomyces produce potent antibacterial substances (Stanier et al.) that could possibly preclude establishment of other bacteria within the niche. The phenomenon of bacterial interference as it affects the respiratory tract of feedlot cattle should be further studied. There is not sufficient evidence in this survey to conclude that the presence of one organism excludes another from the same location.

The numbers of a particular organism that were recovered from a location and statistically determined as colonizing varied from one bacterial genus to another and from one sampling site to another. This was to be expected on the basis of the prevalence of the bacteria in the environment and the non-independence between sampling sites.

It was not possible to identify all organisms as to species. Colonization was therefore determined on the basis of genera of organism. Statistical analysis to determine colonization assumed a Poisson probability model for the randomness of distribution of an organism in a sampling site. This analysis may be in error to the degree that this model was fitted to the distribution of a genus of organism instead of the species.

BACTERIAL FLORA OF BOVINE RESPIRATORY TRACT

by

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ABSTRACT

Bovine respiratory disease is a cause of great economic loss in the cattle industry. Its etiology has been attributed to a combination of complex factors which include viruses, bacteria and environmental stress. Pasteurella sp. are the most frequently recovered bacterial isolate, but its association with apparently healthy cattle lung tissue has not been reported.

The respiratory tract of 50 cattle were examined at slaughter for aerobic bacteria. Cotton tipped applicators were used to obtain tracheal and bronchial fluids from ten locations in each lung. Two locations in the trachea and eight locations corresponding with the distal bronchi of the major pulmonary lobes were examined. Four or five selective and differential media were used for primary isolation.

A total of 433 isolates comprising 22 genera were recovered. Streptomyces sp. were the most frequently recovered and represented 22.7% of the total isolates. Pasteurella sp. represented 7.8% of all isolates and were recovered from 18 lungs. They were considered transient in nine and colonizing in nine.

The trachea and tracheal bifurcation were most frequently colonized by bacteria. There was no significant difference between levels of colonization of different lobes of the lungs. Pasteurella sp. colonized the trachea and tracheal bifurcation more frequently than the bronchi. It was hypothesized that physical stress to which animals were subjected prior to slaughter may be responsible for colonization of bronchi by pasteurellae.

It was concluded that the respiratory tract of apparently healthy beef cattle was not a sterile environment. The region from the trachea to the distal bronchi were colonized by various species of bacteria, most of which had their origin in the soil, feces or pharynx. These organisms persisted in spite of the elaborate mechanism by which the lung rids itself of particulate matter. This mechanism was not adequate to maintain sterility. It was not

certain whether this inadequacy was due to an inherent defect that was peculiar to beef cattle or due to the presence of an overwhelming number of organisms in inhaled air. It is recommended that the pulmonary defense mechanisms in feedlot cattle be further studied.

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APPENDICES

CALCULATION USED TO DETERMINE COLONIZATION

As an illustrative example of the procedure used in determining colonization, suppose that in the tracheal bifurcation, the following number of corynebacterium isolates were observed in 50 respiratory tracts.

X_i =	0	1	2	3	4	5	6	7	8	9	10
Observed frequency f_i =	37	5	3	2	0	1	0	0	0	1	1
Relative frequency m_i =	.74	.10	.06	.04	0	.02	0	0	0	.02	.02

This particular sample had a mean $\bar{x}=0.82$ isolates per respiratory tract at the given site. The generalized Poisson model is (Cohen, 1960):

$$\Pr(X_o=0) = e^{-\theta}(1-e\lambda)$$

$$\Pr(X_1=1) = \theta e^{-\theta}(1-\lambda)$$

$$\Pr(X_j=j) = \theta^j e^{-\theta}/j! \text{ for } j \geq 2$$

$e = 2.71828$ is the base of natural logarithms.

θ and λ can be estimated from the sample as follows:

$$\hat{\theta} = \tfrac{1}{2}[\bar{x}-1+m_o + \{(\bar{x}-1+m_o)^2+4(\bar{x}-m_1)\}^{\frac{1}{2}}] \text{ and}$$

$$\hat{\lambda} = (m_o-m_1\hat{\theta}-1)(m_o+m_1)^{-1}.$$

For example, this becomes

$$\hat{\theta} = 1.174, \quad \hat{\lambda} = 0.78$$

and for X_1 =	0	1	2	3	4	>4
$\Pr(X_i=i)$ =	.592	0.08	0.213	0.083	0.024	.008
F_i =	29.6	4.0	10.7	4.2	1.2	0.4

Where $F_i = \Pr(X_i=i)\ 50$ is the expected frequency.

To test goodness of fit, a chi-square procedure is applied:

$$X^2 = \sum_{i=o}^{k} \frac{(f_i - F_i)}{F_i} \text{ with V degrees of freedom.}$$

In this test, the classes X_4 and $X>_4$ are lumped together so that no class has an $F_i<1$. Therefore, $V=5-3=2$. (In these chi-square tests, the degrees of freedom are always the number of classes with

$F_i \geq 1$ less 3).

The chi-square value calculated is 11.82 which is sufficently large to reject the hypothesis that the model fits the data observed.

The next step then is to eliminate the class X_{10} and repeat the procedure. Again, a sufficiently high chi-square value is observed to reject the goodness of fit hypothesis. Next, the X_9 class is eliminated along with all contiguous classes having an $f_i = 0$. This leaves

X_i =	0	1	2	3	4	5
f_i =	37	5	3	2	0	1

Computing $\hat{\theta} = .720$, $\hat{\lambda} = .716$, we find

X_i =	0	1	2	3	4	5
Pr $(X_i=i)$ =	.738	.100	.126	.030	.005	.001
F_i =	35.4	4.8	6.1	1.5	.3	0

In order for the chi-square test to be unbiased, the X_3, X_4 and X_5 classes are lumped together. The value of the test then is calculated as 2.46 with one degree of freedom which is not large enough to reject the hypothesis of goodness of fit, that is, the model is adequate to describe this subset of the data. Although a better fit would result if the data were further truncated to say X_3 it is unnecessary. Note that $P(X_5=5) < .001$ and this is sufficient to adopt the rule that any $X \geq 5$ provides positive evidence of non randomness, i.e. colonization of Corynebacterium in the tracheal bifurcation.

Table 1. Aerobic bacteria isolated from Lung No. 1

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces	2
Tracheal bifurcation	Streptomyces	1
	Baccillus subtilis	1
	Staphylococcus simulans	1
	Streptococcus (Group F)	1
	Pasturella hemolytica	1
	Pseudomonas putida	2
Right cranial apical lobe	Corynebacterium sp.	1
	Staphylococcus epidermidis	1
Right caudal apical lobe	Streptomyces	1
Cardiac lobe	Streptococcus (Group D)	1
	Streptomyces	1
Right diaphramatic lobe	Corynebacterium sp.	13
Accessory lobe	Streptomyces	3
	Staphylococcus epidermidis	12
	Streptococcus (Group D)	2
	Neisseria sp.	2
Left cranial apical lobe	Streptomyces	2
Left caudal apical lobe	Streptomyces	1
Left diaphramatic lobe	Streptomyces	1

Table 2. Aerobic bacteria isolated from Lung No. 2

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Bacillus subtilis	1
	Neisseria sp.	TNTC
	Pasteurella hemolytica	TNTC
Tracheal bifurcation	Streptomyces sp.	5
	Staphylococcus xylosus	2
	Neisseria sp.	4
	Pasteurella hemolytica	1
Right cranial apical lobe	Streptomyces	2
Right caudal apical lobe	-	-
Cardiac lobe	Streptomyces	1
Right diaphramatic lobe	Streptomyces	2
Accessory lobe	Streptomyces	2
	Bacillus pumilus	1
	Streptococcus (Group D)	1
Left cranial apical lobe	Staphylococcus epidermidis	12
	Streptococcus (Group D)	4
	Neisseria sp.	1
Left caudal apical lobe	Staphylococcus epidermidis	2
	Neisseria sp.	1
	Pasteurella hemolytica	3
Left diaphramatic lobe	Staphylococcus epidermidis	1

TNTC = Too numerous to count (Indicates a count of over 90 colony forming units)

Table 3. Aerobic Bacteria isolated from Lung No. 3

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	TNTC
	Baccillus firmus	1
	Corynebacterium sp.	9
	Staphylococcus epidermidis	20
	Micrococcus sp.	3
	Streptococcus (Group D)	5
	Branhamella catarrhalis	2
	Pasteurella hemolytica	4
	Pseudomonas testosteroni	2
	Escheriohia coli	4
Tracheal Bifurcation	Streptomyces sp.	TNTC
	Baccillus subtilis	2
	Micrococcus sp.	15
	Streptococcus (Group D)	17
Right cranial apical lobe	Streptomyces sp.	30
	Corynebacteriums sp.	28
	Streptococcus (Group B)	1
	Staphylococcus epidermidis	11
	Moraxella sp.	5
	Neisseria sp.	4
	Group IIo	16
Right caudal apical lobe	Streptomyces sp.	25
	Micrococcus sp.	2
Cardiac lobe	Streptomyces sp.	35
Right diaphramatic lobe	Streptomyces sp.	TNTC
Accessory lobe	Streptomyces sp.	TNTC
Left cranial apical	Streptomyces sp.	TNTC
	Staphylococcus epidermidis	1
Left caudal apical	Streptomyces sp.	98
	Streptococcus (Group B)	1
Left diaphramatic lobe	Streptomyces sp.	TNTC

TNTC = Too numerous to count (Indicates a count of over 90 colony forming units)

Table 4. Aerobic bacteria isolated from Lung No. 4

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp. Corynebacterium sp.	TNTC 2
Tracheal bifurcation	Streptomyces sp. Escherichia coli	TNTC 2
Right cranial apical lobe	Streptomyces sp.	55
Right caudal apical lobe	Streptomyces sp.	90
Cardiac lobe	Streptomyces sp.	TNTC
Right diaphramatic lobe	Streptomyces sp.	TNTC
Accessory lobe	Streptomyces sp.	TNTC
Left cranial apical lobe	Streptomyces sp.	TNTC
Left caudal apical lobe	Streptomyces sp.	TNTC
Left diaphramatic lobe	Streptomyces sp.	TNTC

TNTC = Too numerous to count (Indicates a count of over 90 colony forming units)

Table 5. Aerobic bacteria isolated from Lung No. 5

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	1
	Staphylococcus hominis	2
	Micrococcus sp.	8
	Streptococcus (Group F)	12
	Moraxella sp.	1
	Acinetobacter calcoaceticus variety calcoaceticus	4
	Pasteurella multocida	2
	Pseudomonas aeruginosa	2
Tracheal bifurcation	Staphylococcus hominis	2
	Streptococcus (Group F)	5
	Moraxella sp.	2
	Neisseria sp.	1
Right cranial apical lobe	Streptococcus (Group F)	1
Right caudal apical lobe	Neisseria sp.	1
	Pseudomonas aeruginosa	1
Cardiac lobe	Micrococcus sp.	2
Right diaphramatic lobe	-	-
Accessory lobe	Streptomyces	1
Left cranial apical lobe	Streptomyces	2
	Neisseria sp.	1
Left caudal apical lobe	Staphylococcus hominis	1
	Neisseria sp.	4
Left diaphramatic lobe	Micrococcus	4
	Streptococcus Group F	3

Table 6. Aerobic bacterial flora isolated from Lung No. 6

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	Streptomyces	4
	Micrococcus sp.	2
	Streptococcus (Group D)	1
Tracheal bifurcation	Neisseria sp.	1
Right cranial apical lobe	-	-
Right caudal apical lobe	Streptomyces	2
Cardiac lobe	Streptomyces	1
Right diaphramatic lobe	Baccillus pumilis	2
Accessory lobe	Streptomyces sp.	2
	Staphylococcus epidermidis	2
	Micrococcus sp.	1
	Streptococcus (Group D)	1
Left cranial apical lobe	Corynebacterium sp.	1
	Staphlococcus epidermidis	8
	Streptococcus (Group D)	5
Left caudal apical lobe	-	-
Left diaphramatic lobe	Streptomyces	3

Table 7. Aerobic Bacteria isolated from Lung No. 7

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	1
	Staphylococcus epidermidis	1
	Streptococcus(Group F)	1
Tracheal bifurcation	Corynebacterium sp.	1
Right cranial apical lobe	-	-
Right caudal apical lobe	Streptococcus (Group F)	2
	Streptococcus (Group B)	1
	Neisseria sp.	1
Cardiac lobe	-	-
Right diaphramatic lobe	Corynebacterium sp.	1
	Neisseria sp.	1
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	Corynebacterium sp.	1
Left diaphramatic	Neisseria sp.	1

Table 8. Aerobic bacteria isolated from Lung No. 8

Site in the respiratory tract	Name of bacteria	Number of colony forming units
Trachea	Streptomyces sp.	3
	Streptococcus (Group B)	2
Tracheal bifurcation	Corynebacterium sp.	1
Right cranial apical lobe	Micrococcus sp.	1
Right caudal apical lobe	-	-
Cardiac lobe	Corynebacterium sp.	1
Right diaphramatic lobe	Micrococcus sp.	1
Accessory lobe	Corynebacterium sp.	1
	Streptococcus (Group B)	1
Left cranial apical lobe	Baccillus sphericus	1
	Corynebacterium sp.	1
Left caudal apical lobe	Staphylococcus warneri	1
	Neisseria mucosa	2
Left diaphramatic lobe	-	-

Table 9. Aerobic Bacteria isolated from Lung No. 9

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	Streptomyces sp.	3
	Baccillus laterosporus	1
	Corynebacterium sp.	1
	Staphylococcus simulans	2
	Streptococcus (Group A)	2
	Streptococcus (Group B)	1
	Acinetobacter calcoacelicus variety calcoaceticus	2
	Pasteurella hemolytica	1
Tracheal bifurcation	Streptomyces sp.	3
	Streptococcus (Group D)	4
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	Streptococcus (Group B)	1
	Neisseria sp.	1
Right diaphramatic	Streptomyces sp.	2
	Micrococcus sp.	1
Accessory lobe	Staphylococcus simulans	1
Left cranial apical lobe	Streptomyces sp.	1
Left caudal apical lobe	-	-
Left diaphramatic	Streptomyces	1

Table 10. Aerobic Bacteria isolated from Lung No. 10

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	1
	Corynebacterium sp.	1
	Staphylococcus simulans	3
	Streptococcus Group D	1
	Pasteurella hemolytica	30
Tracheal bifurcation	Streptomyces sp.	2
	Corynebacterium sp.	1
	Staphylococcus xylosus	1
	Streptococcus Group D	3
Right cranial apical lobe	-	-
Right caudal apical lobe	Streptomyces sp.	1
	Baccillus pumilis	1
Cardiac lobe	Streptomyces sp.	1
Right diaphramatic lobe	Streptomyces	2
	Staphylococcus xylosus	1
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	Streptomyces	1
	Staphylococcus xylosus	1

Table 11. Aerobic bacteria isolated from Lung No. 11

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	2
	Staphylococcus cohnii	2
Tracheal bifurcation	Streptomyces sp.	1
	Corynebacterium sp.	1
	Staphylococcus cohnii	1
	Pasteurella hemolytica	1
	Haemophilus-like	17
Right cranial apical lobe	-	-
Right caudal apical lobe	Baccillus pumilis	1
Cardiac lobe	Streptomyces sp.	2
Right diaphramatic lobe	Corynebacterium sp.	1
	Pasteurella hemolytica	8
	Group IIF	1
Accessory lobe	Staphylococcus epidermidis	10
	Bordetella	1
Left cranial apical lobe	Staphylococcus epidermidis	8
	Acinetobacter calcoaceticus variety calcoaceticus	1
	Group IIF	2
Left caudal apical lobe	Streptomyces sp.	6
	Staphylococcus epidermidis	2
Left diaphramatic	Streptomyces sp.	5
	Staphylococcus epidermidis	1
	Hemophilus-like	TNTC

TNTC = Too numerous to count (Indicates a count of over 90 colony forming units).

Table 12. Aerobic bacteria isolated from Lung No. 12

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	Staphylococcus epidermidis	22
Tracheal bifurcation	Streptomyces sp.	5
Right cranial apical lobe	-	-
Right caudal apical lobe	Corynebacterium sp.	1
	Staphylococcus epidermidis	2
	Acinetobacter calcoaceticus variety Lwoffi	3
Cardiac lobe	Acinetobacter calcoaceticus variety Lwoffi	2
Right diaphramatic lobe	Staphylococcus epidermidis	1
	Pasteurella sp.	1
Accessory lobe	-	-
Left cranial apical	Staphylococcus epidermidis	2
	Acinetobacter calcoaceticus variety Lwoffi	1
Left caudal apical lobe	Baccillus subtilis	1
	Streptococcus Group D	1
Left diaphramatic	Streptomyces sp.	2
	Streptococcus Group D	1

Table 13. Aerobic bacteria isolated from Lung No. 13

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	1
	Staphylococcus hominis	1
	Neisseria sp.	4
Tracheal bifurcation	Neisseria sp.	1
Right cranial apical lobe	Staphylococcus hominis	1
	Enterobacter liquefiaciens	1
Right caudal apical lobe	Acinetobacter calcoaceticus variety calcoaceticus	1
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	Pseudomonas aeruginosa	1
	Enterobacter liquefiaciens	11
Left cranial apical lobe	Staphylococcus epidermidis	1
Left caudal apical lobe	-	-
Left diaphramatic	Staphylococcus epidermidis	1
	Neisseria sp.	3
	Enterobacter liquefiaciens	3

Table 14. Aerobic bacteria isolated from lung No. 14

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	Streptomyces sp. Pasteurella hemolytica	1 16
Tracheal bifurcation	Streptomyces sp. Streptococcus Group B	6 1
Right cranial apical lobe	Streptomyces sp.	1
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	Streptomyces sp. Neisseria sp.	1 2
Accessory lobe	Streptomyces sp.	2
Left cranial apical	-	-
Left caudal apical	-	-
Left diaphramatic lobe	Corynebacterium sp. Aeromonas hydrophila	3 TNTC

TNTC = Too numerous to count (Indicates a count of over 90 colony forming units)

Table 15. Aerobic bacteria isolated from Lung No. 15

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	3
	Staphylococcus hominis	1
	Neisseria sp.	3
	Enterobacter liquefiaciens	1
Tracheal bifurcation	Staphylococcus hominis	1
	Streptococcus Group D	2
Right cranial apical lobe	-	-
Right caudal apical lobe	Streptomyces sp.	1
	Micrococcus	1
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	Streptomyces sp.	1
Left caudal apical lobe	Streptomyces sp.	2
	Enterobacter liquefiaciens	2
Left diaphramatic lobe	Enterobacter liquefiaciens	5

Table 16. Aerobic bacteria isolated from Lung No. 16

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	Baccillus subtilis	1
	Corynebacterium sp.	3
	Staphylococcus werneri	4
	Streptococcus Group C	3
	Pasteurella multocida	20
Tracheal bifurcation	Corynebacterium sp.	2
	Pasteurella multocida	10
Right cranial apical lobe	-	-
Right caudal apical lobe	Baccillus subtilis	3
Cardiac lobe	Enterobacter liquefiaciens	1
Right diaphramatic lobe	Moraxella sp.	2
Accessory lobe	-	-
Left cranial apical lobe	Staphylococcus werneri	3
Left caudal apical lobe	Baccillus subtilis	4
	Corynebacterium sp.	1
Left diaphramatic lobe	-	-

Table 17. Aerobic bacteria isolated from Lung No. 17

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	Flavobacterium sp.	1
Tracheal bifurcation	Streptomyces sp. Corynebacterium sp.	1 1
Right cranial apical lobe	-	-
Right caudal apical lobe	Streptomyces	1
Cardiac lobe	-	-
Right diaphramatic lobe	Baccillus subtilis	1
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 18. Aerobic bacteria isolated from Lung No. 18

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	-	-
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic	Streptomyces	1
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	Streptomyces sp.	-

Table 19. Aerobic bacteria isolated from Lung No. 19

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	1
Tracheal bifurcation	Moraxella sp.	20
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	Baccillus pumilis Pasteurella multocida	1 1
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	Baccillus pumilis Pasteurella multocida	1 1

Table 20. Aerobic Bacteria isolated from Lung No. 20

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	Corynebacterium sp.	1
Tracheal bifurcation	-	-
Right cranial apical lobe	Baccillus subtilis	1
	Corynebacterium sp.	4
	Staphylococcus epidermidis	1
	Group IVF	2
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	Baccillus pumilis	2
Left cranial apical lobe	Neisseria sp.	1
	Pseudomonas maltophilia	1
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 21. Aerobic bacteria isolated from lung No. 21

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Staphylococcus epidermidis	1
	Streptomyces	3
	Streptococcus Group B	3
	Neisseria sp.	1
	Citrobacter freundii	1
Tracheal bifurcation	Streptomyces sp.	10
	Streptococcus Group D	1
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	Streptomyces sp.	4
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic	Pseudomonas acidovorans	5
	Group IIB	2

Table 22. Aerobic bacteria isolated from Lung No. 22

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	2
	Baccillus pumilis	1
	Corynebacterium sp.	3
	Neisseria sicca	2
	Pasteurella gallinarium	2
Tracheal bifurcation	Streptomyces sp.	2
Right cranial apical lobe	-	-
Right caudal apical lobe	Baccillus pumilis	1
	Corynebacterium sp.	1
Cardiac lobe	-	-
Right diaphramatic lobe	Staphylococcus werneri	1
	Streptococcus Group D	10
	Neisseria sicca	10
Accessory lobe	Streptomyces sp.	1
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	Corynebacterium sp.	1
	Neisseria sicca	1

Table 23. Aerobic bacteria isolated from Lung No. 23

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	50
	Baccillus subtilis	1
	Streptococcus Group B	2
Tracheal bifurcation	Streptomyces sp.	5
	Streptococcus Group D	2
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	Streptomyces sp.	3
Accessory lobe	Streptomyces sp.	5
Left cranial apical lobe	Streptomyces sp.	2
Left caudal apical lobe	-	-
Left diaphramatic lobe	Baccillus circulans	1

Table 24. Aerobic bacteria isolated from Lung No. 24

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	Streptomyces sp.	10
	Pasteurella hemolytica	1
	Pseudomonas dimunita	1
Tracheal bifurcation	Streptomyces sp.	5
	Staphylococcus epidermidis	2
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	Streptomyces sp.	2
Right diaphramatic lobe	Streptomyces sp.	11
	Baccillus subtilis	1
Accessory lobe	-	-
Left cranial apical lobe	Streptomyces sp.	2
Left caudal apical lobe	-	-
Left diaphramatic lobe	Streptomyces	6

Table 25. Aerobic bacteria isolated from Lung No. 25

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	Streptomyces sp. Moraxella osloensis	1 5
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 26. Aerobic bacteria isolated from Lung No. 26

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Hemophilus-like	TNTC
Tracheal bifurcation	Hemophilus-like	TNTC
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

TNTC = Too numerous to count (Indicates a count of over 90 colony forming units).

Table 27. Aerobic bacteria isolated from Lung No. 27

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Corynebacterium sp.	2
Tracheal bifurcation	Baccillus pumilis Neisseria sp.	1 1
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	Corynebacterium sp.	5
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 28. Aerobic bacteria isolated from Lung No. 28

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	4
	Baccillus pumilis	2
	Corynebacterium sp.	5
	Neisseria sp.	3
Tracheal bifurcation	Streptomyces sp.	6
	Baccillus pumilis	2
	Corynebacterium sp.	1
	Neisseria sp.	9
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	Streptomyces sp.	2
	Corynebacterium sp.	4
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	Neisseria sp.	2

Table 29. Aerobic bacteria isolated from Lung No. 29

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	Moraxella osloensis	2
Tracheal bifurcation	Baccillus stearothermophilus Micrococcus sp.	5 2
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	Micrococcus sp.	1
Left diaphramatic lobe	-	-

Table 30. Aerobic bacteria isolated from Lung No. 30

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Micrococcus sp.	2
Tracheal bifurcation	Baccillus circulans	1
Right cranial apical lobe	-	-
Right caudal apical lobe	Baccillus stearothermophilus Baccillus subtilis	1 1
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 31. Aerobic bacteria isolated from Lung No. 31

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Corynebacterium sp.	2
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	Baccillus stearothermophilus	1
Cardiac lobe	-	-
Right diaphramatic lobe	Baccillus stearothermophilus	2
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 32. Aerobic bacteria isolated from Lung No. 32

Site in respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	-	-
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	Corynebacterium sp. Streptococcus Group D	6 1
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	Micrococcus sp.	1
Left diaphramatic lobe	-	-

Table 33. Aerobic bacteria isolated from Lung No. 33

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Pasteurella multocida	TNTC
Tracheal bifurcation	Pasteurella multocida	TNTC
Right cranial apical lobe	Acinetobacter calcoaceticus variety Lwoffi Pseudomonas testosteroni	1 1
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	Pasteurella multocida	TNTC
Left cranial apical lobe	-	-
Left caudal apical lobe	Pasteurella multocida	1
Left diaphramatic lobe	Baccillus laterosporus	2

TNTC = Too numerous to count (Indicates a count of more than 90 colony-forming units)

Table 34. Aerobic bacteria isolated from Lung No. 34

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	Corynebacterium sp.	10
	Staphylococcus xylosus	TNTC
	Micrococcus sp.	7
	Moraxella osloensis	TNTC
Tracheal bifurcation	Moraxella osloensis	TNTC
Right cranial apical lobe	Pseudomonas maltophilia	2
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

TNTC = Too numerous to count (Indicates a count of over 90 colony forming units).

Table 35. Aerobic bacteria isolated from Lung No. 35

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Moraxella osloensis	1
Tracheal bifurcation	Staphylococcus xylosus Moraxella osloensis	1 10
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 36. Aerobic bacteria isolated from Lung No. 36

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Corynebacterium sp. Micrococcus sp.	1 4
Tracheal bifurcation	-	-
Right cranial apical lobe	Corynebacterium sp. Group IIF	1 1
Right caudal apical lobe	Baccillus pumilus	1
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	Corynebacterium sp.	2
Left caudal apical lobe	-	-
Left diaphramatic lobe	Baccillus pumilus	1

Table 37. Aerobic bacteria isolated from Lung No. 37

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	Baccillus subtilis	2
	Corynebacterium sp.	1
Tracheal bifurcation	Streptomyces sp.	1
	Baccillus pumilus	2
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	Corynebacterium sp.	1
Left cranial apical lobe	Escherichia coli	1
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 38. Aerobic bacteria isolated from Lung No. 38

Site in the respiratory tract	Name of Bacteria	Number of colony forming units
Trachea	-	-
Tracheal bifurcation	Corynebacterium sp. Lactobaccillus sp.	1 1
Right cranial apical lobe	Baccillus megatarium Micrococcus sp.	1 1
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 39. Aerobic bacteria isolated from Lung No. 39.

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	-	-
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Note - No organism was isolated from any of the examined locations in Lung No. 39.

Table 40. Aerobic bacteria isolated from Lung No. 40

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Baccillus subtilis Micrococcus sp.	1 2
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	Corynebacterium sp.	3
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 41. Aerobic bacteria isolated from Lung No. 41

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Streptomyces sp.	2
	Moraxella liquefiaciens	1
Tracheal bifurcation	Corynebacterium sp.	3
	Moraxella liquefiaciens	3
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 42. Aerobic bacteria isolated from Lung No. 42

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	-	-
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	Corynebacterium sp. Moraxella osloensis	2 5

Table 43. Aerobic bacteria isolated from Lung 43

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	-	-
Tracheal bifurcation	Lactobaccillus sp.	50
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 44. Aerobic bacteria isolated from Lung No. 44

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	-	-
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Note - No organism was isolated from any of the locations examined in Lung No. 44.

Table 45. Aerobic bacteria isolated from Lung No. 45

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	-	-
Tracheal bifurcation	Streptomyces sp. Baccillus subtilis	1
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	Micrococcus sp.	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 46. Aerobic bacteria isolated from Lung Number 46

Site in the respiratory tract	Name of bacteria	Number of Colony forming units
Trachea	-	-
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	Corynebacterium sp.	2
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	Micrococcus sp.	2
Left diaphramatic lobe	-	-

Table 47. Aerobic bacteria isolated from Lung No. 47

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Baccillus pumilis	4
	Pasteurella multocida	3
Tracheal bifurcation	Micrococcus sp.	9
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	-	-
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	Corynebacterium sp.	3
	Micrococcus sp.	4
Left diaphramatic lobe	-	-

Table 48. Aerobic bacteria isolated from Lung No. 48

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Branhamella catarrhalis	17
	Pasteurella hemolytica	TNTC
Tracheal bifurcation	Branhamella catarrhalis	TNTC
	Pasteurella hemolytica	TNTC
Right cranial apical lobe	Pasteurella hemolytica	5
Right caudal apical lobe	Pasteurella hemolytica	26
Cardiac lobe	Pasteurella hemolytica	30
Right diaphramatic lobe	-	-
Accessory lobe	Branhamella catarrhalis	5
	Pasteurella hemolytica	TNTC
Left cranial apical lobe	-	-
Left caudal apical lobe	-	-
Left diaphramatic lobe	Pasteurella hemolytica	2

TNTC = Too numerous to count (Indicates a count of over 90 colony forming units).

Table 49. Aerobic bacteria isolated from Lung No. 49

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	Pasteurella hemolytica	7
Tracheal bifurcation	Neisseria sp. Pasteurella hemolytica	5 7
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	Baccillus pumilis	1
Right diaphramatic lobe	Baccillus subtilis	1
Accessory lobe	Corynebacterium sp. Acinetobacter calcoaceticus variety calcoaceticus	5 2
Left cranial apical lobe	Acinetobacter calcoaceticus variety calcoaceticus Pasteurella hemolytica	1 1
Left caudal apical lobe	-	-
Left diaphramatic lobe	-	-

Table 50. Aerobic bacteria isolated from Lung No. 50

Site in the respiratory tract	Name of Bacteria	Number of Colony forming units
Trachea	-	-
Tracheal bifurcation	-	-
Right cranial apical lobe	-	-
Right caudal apical lobe	-	-
Cardiac lobe	Micrococcus sp.	1
Right diaphramatic lobe	-	-
Accessory lobe	-	-
Left cranial apical lobe	-	-
Left caudal apical lobe	Corynebacterium sp. Micrococcus sp. Moraxella sp.	29 1 2
Left diaphramatic lobe		

BACTERIAL FLORA OF BOVINE RESPIRATORY TRACT

by

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ABSTRACT

Bovine respiratory disease is a cause of great economic loss in the cattle industry. Its etiology has been attributed to a combination of complex factors which include viruses, bacteria and environmental stress. Pasteurella sp. are the most frequently recovered bacterial isolate, but its association with apparently healthy cattle lung tissue has not been reported.

The respiratory tract of 50 cattle were examined at slaughter for aerobic bacteria. Cotton tipped applicators were used to obtain tracheal and bronchial fluids from ten locations in each lung. Two locations in the trachea and eight locations corresponding with the distal bronchi of the major pulmonary lobes were examined. Four or five selective and differential media were used for primary isolation.

A total of 433 isolates comprising 22 genera were recovered. Streptomyces sp. were the most frequently recovered and represented 22.7% of the total isolates. Pasteurella sp. represented 7.8% of all isolates and were recovered from 18 lungs. They were considered transient in nine and colonizing in nine.

The trachea and tracheal bifurcation were most frequently colonized by bacteria. There was no significant difference between levels of colonization of different lobes of the lungs. Pasteurella sp. colonized the trachea and tracheal bifurcation more frequently than the bronchi. It was hypothesized that physical stress to which animals were subjected prior to slaughter may be responsible for colonization of bronchi by pasteurellae.

It was concluded that the respiratory tract of apparently healthy beef cattle was not a sterile environment. The region from the trachea to the distal bronchi were colonized by various species of bacteria, most of which had their origin in the soil, feces or pharynx. These organisms persisted in spite of the elaborate mechanism by which the lung rids itself of particulate matter. This mechanism was not adequate to maintain sterility. It was not

certain whether this inadequacy was due to an inherent defect that was peculiar to beef cattle or due to the presence of an overwhelming number of organisms in inhaled air. It is recommended that the pulmonary defense mechanisms in feedlot cattle be further studied.