

The Balance of Airborne Bacteria in Calf Houses

C. M. WATHES; K. HOWARD; C. D. R. JONES; A. J. F. WEBSTER*

The bacterial content of the air and physical environment of two crated veal calf units were monitored over the growing period of 16 weeks. The rate of release of bacteria colony forming particles (BCFP) from the calves was rapid, typically 2×10^6 BCFP/h per unit calf area, and showed a positive correlation with absolute humidity. The concentration of airborne BCFP arising mainly from these calves, was about 30 BCFP/l, but was not a simple function of climate. In addition to clearance by ventilation at rates ranging from 2 to 10 air changes/h the airborne bacteria were removed by sedimentation and other physical processes, and killed by normal biological mechanisms. The combined rate for the latter two pathways of clearance was equivalent to 4-49 air changes/h, which is of a magnitude comparable with ventilation rates in naturally-ventilated livestock buildings.

At present the critical concentrations of non-pathogenic bacteria in animal houses are unknown and so a minimum ventilation rate based on this criterion cannot be calculated. However recognition of the different routes of clearance of airborne bacteria, including ventilation, is fundamental to the design of animal houses.

1. Introduction

A farm animal confined indoors breathes air polluted by inert dusts, microbial aerosols and noxious gases at concentrations much higher than those outdoors. Clearance of these by-products of the existence of the animal is one important function of ventilation.¹ Although dust has been implicated in the aetiology of some respiratory diseases,² e.g. chronic bronchitis in the horse, there is apparently little correlation between airborne dust levels and the performance of housed animals.⁴ Similarly, high concentrations of toxic or irritant gases or of non-pathogenic microorganisms may impair pulmonary clearance leading to pneumonia in calves⁵ but there is little experimental evidence for this controversial hypothesis. Air pollutants may indeed harm the health of an animal,⁶ but the critical levels for chronic exposure to such agents, both singly and in combination are still unknown.⁷

The traditional criteria by which the rate of ventilation is set are the temperature and humidity of the air and the levels of carbon dioxide and oxygen.⁸ Humidity is controlled to prevent surface condensation while air temperature is manipulated according to the thermal regulatory and physiological responses of the animal. In both cases the rates of production and removal (of heat and moisture) are known accurately and the supply of "fresh" air is adjusted to maintain target temperatures and humidities. A steady-state balance equation is often used for the prediction of equilibrium concentrations. Therefore, if a building designer is to employ the level of airborne bacteria as a criterion for ventilation he must first understand their kinetics.

The purpose of this paper is (1) to describe a simple model of airborne microbes and bacteria in a calf house as an example; (2) to present measurements of the release and clearance rates of aerobic bacteria and (3) to review the importance of bacterial aerosols in the aetiology of respiratory diseases of housed animals in general and calves in particular.

*Department of Animal Husbandry, University of Bristol, School of Veterinary Science, Langford, Bristol BS18 7DU

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2. Theory

mass balance for a pollutant flow into and out of a building is

$$\frac{dC}{dt} = \frac{R}{V} - q_e C + q_v(C_a - C), \quad \dots(1)$$

C is the indoor concentration, t is time, V is the volume of the building, R is the emission rate, C_a is the ambient concentration, q_v is the air change rate and q_e is the sum of the specific rate constants and represents clearance from the air by mechanisms other than ventilation. The solution of Eqn (1) is

$$C = C_0 e^{-(q_e + q_v)t} + \frac{R + Vq_v C_a}{V(q_e + q_v)} (1 - e^{-(q_e + q_v)t}), \quad \dots(2)$$

C_0 is the initial pollutant concentration at $t=0$. Eqn (2) applies to a one-compartment building with a constant emission rate and assumes complete mixing within the air space. Complex multi-compartment models of air flows, which may be appropriate for some livestock buildings, have been developed⁹ and the consequences of incomplete mixing are now recognized.^{10,11} In most cases $q_e + q_v$ exceeds 10/h and the exponential term becomes negligible after 15 min or so, giving the familiar expression for the equilibrium pollutant concentration.

$$C = \frac{R + Vq_v C_a}{V(q_e + q_v)}. \quad \dots(3)$$

In the special case when R and q_e are constant and $C \gg C_a$, Eqn (3) can be rearranged to give a relationship between q_v and $1/C$:

$$1/C = a + b q_v, \quad \dots(4)$$

where $a = V q_e/R$ and $b = V/R$. R and q_e can then both be determined from a plot of $1/C$ versus q_v . Alternatively, if R , C and q_v are measured, q_e can be calculated directly from Eqn (3).

The units of q_e are the same as q_v and q_e can be thought of as an equivalent rate of air change for clearance by other pathways. The advantages of representing clearance rates in this way are twofold. First, the combined effect of several processes occurring simultaneously is simply proportional to the sum of the rate constants and, second, the rates are independent of pollutant concentration.¹²

The major sink for airborne gases is ventilation; inhalation by the animal is a minor route of clearance and q_e is effectively zero. However, for microbial aerosols and dust several other mechanisms are important. Sedimentation, impaction and deposition on horizontal surfaces occur at a rate proportional to the particle density and size; typical rate constants for sedimentation are, respectively, 0.1/h and 10/h for 1 μm and 10 μm unit density particles dispersed at 1 m height in still air. The survival of airborne microbes depends,¹³ amongst other things, on temperature, humidity and composition of the suspending fluid with death rate constants ranging from 0.1/h to 100/h for individual species of bacteria.¹⁴ Finally, the effectiveness of mechanical devices such as scrubbers, filters and ionizers can be considered in the above terms and will obviously depend on the efficiency of the machine and its throughput.

3. Experimental methods

3.1. Calves and calf housing

The Friesian bull calves were housed in January 1983 at approximately 1 week of age in individual wooden crates in each of two rooms, and were reared as veal calves until slaughter at

17 weeks of age. The calves were also the subject of a nutritional trial but a similar ration was allocated to each room. Each animal was weighed weekly and records of its health and veterinary treatment were kept. In all other respects the husbandry of the calves followed conventional practices, but with particular attention paid to daily cleansing and manure disposal.

The layout and dimensions of the rooms are shown in Fig. 1. The room volume was 110 m³ giving a cubic capacity of 12 m³ per calf. Each room was ventilated naturally; air entered through a narrow inlet, 1.58 × 0.15 m, positioned at eaves height and was exhausted through a 0.6 m² opening in the roof apex containing a propeller fan, the motor of which was disconnected from its power supply but which could free-wheel when naturally induced air flow passed through the fan blades. An air dehumidifier was installed 2.6 m above the floor in each room. Air was drawn through the unit by a fan with a throughput of 1700 m³/h at 290 Pa pressure difference, and the latent heat of condensation was returned to the room air. The unit was controlled by a humidistat. The rapid flow rate through the unit, equivalent to 15 air changes per hour, ensured that the air in the room was well mixed. In the room which was used as the experimental control, the compressor was disconnected so that the unit recirculated air without dehumidification. The room with the operating humidifier was given three regimes, namely two with controlled conditions, nominally 50% and 70% r.h. and one following ambient as for the control room. These treatments commenced when the calves were 11 days old.

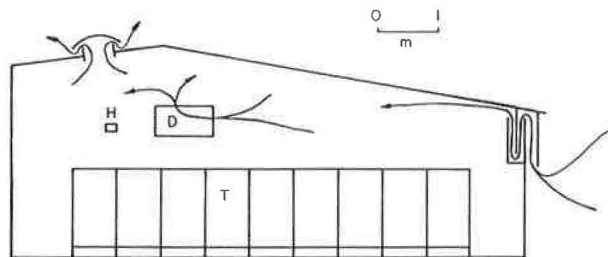


Fig. 1. Cross-sectional view of calf houses. D, Dehumidifier; H, humidistat; T, psychrometer

3.2. Measurements of the physical and aerial environment

Throughout the experiment air temperature and relative humidity were monitored at hourly intervals using a psychrometer with matched thermistors aspirated by a small fan located 1.8 m above the floor at the centre of each room. Ventilation rates and the concentrations of airborne bacteria colony-forming particles (BCFP) were measured daily during alternate weeks after one week of equilibrium at each climate. Air change rates were assessed by recording the clearance of a small sample of a tracer gas, sulphur hexafluoride, using a gas chromatograph. The concentration of airborne BCFP was measured four times daily, nominally at 1000, 1400 and 1600 h, 1 m above the ground in front of the centre pen using nutrient agar plates in a six-stage Andersen sampler¹⁵ with a sampling time of 20–30 s at a flow rate of 28.3 l/min. The plates were incubated aerobically at 25°C for 3 d and the number of colonies recorded. All results are expressed as the geometric mean of the numbers of bacteria.

3.3. Rate of release of bacteria

The rate of release of aerobic bacteria from the calves was measured using the apparatus shown in Fig. 2. This comprised a conical shaped sampling cup (A) of area 93 cm² through which filtered air (B) was drawn at a flow rate of 28.3 l/min from eight ports. The cup was pressed firmly against the flank of the restrained calf at the right scapula and a good seal was ensured by a flexible rubber gasket (C). The velocity of the incoming air stream was calculated

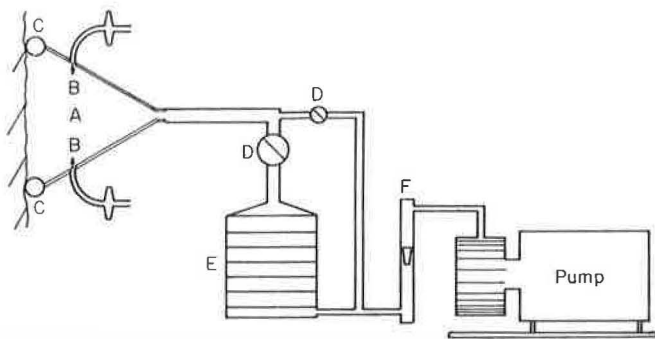


Fig. 2. Schematic diagram of the apparatus for release measurements.

/s, in the same range as the speed of the convection currents from the animal. Prior to measurement, about 7 l of filtered air, controlled by butterfly valves (D), was flushed through apparatus to clear the pipework of any ambient contamination. The sample of air containing bacteria dispersed from the skin and coat of the calf was then passed through the particle sampler (E), as described earlier, with a sampling time of 60–90 s. The flow rate was measured with a flow meter (F).

The release rate from three calves in each room was measured three times on one day of each week. The total release from the calves into the room was calculated from the number of bacteria per body surface area $A = 0.15 W^{0.56}$ where W is the weight of the animal in kg, and A is measured in m^2 .

4. Results

Fig. 3 shows the daily measurements of ventilation rate and the mean air temperature and relative humidity in both rooms. Air temperature rose as the calves aged because of the rising ambient temperatures and was between 2 and 4.3°C hotter in the dehumidified room than in the control room. The relative humidity in the dehumidified room was usually within 5% r.h. of the control room and fluctuated little, with a typical daily standard deviation of 3–4% r.h. Ventilation rates in the two rooms were similar with an overall mean (\pm S.D.) of 3.9 ± 2.09 and 4.4 ± 2.16 air changes/h, respectively.

The mean daily concentrations of airborne bacteria in the dehumidified room was nearly always higher than in the control room except when the compressor was switched off during the ambient temperature rises (Fig. 4). As the calves grew larger the concentration in the control room increased from 6 to 22 BCFP/l at 3 and 16 weeks of age, respectively. The increase in the number of bacteria masked the differential effect of occupancy of the building or climate on the different particle sizes; the concentration of large particles ($>3.5 \mu m$ aerodynamic diameter) rose while that of the small particles ($<3.5 \mu m$) either fell or remained constant, but was substantially lower by a factor of 10 or so. The same underlying trends were observed in the treatment room.

The mean flux of bacteria dispersed by the calves showed an apparent increase with age. This, however, is probably an artefact of the correlation between the climate in the rooms and the age of the calves. Accordingly, the fluxes are shown plotted against absolute humidity ρ , g/m^3 , in Fig. 5. The linear regression of the flux of bacteria R^* , \log_{10} BCFP/ $m^2 h$, on ρ was $R^* = 5.570 + 0.003\rho$ ($n = 30$, $r = 0.74$, $P < 0.001$). R^* was only weakly correlated with relative humidity and temperature ($r = 0.47$, $P < 0.01$; $r = 0.42$, $P < 0.05$, respectively). The fluxes of bacteria were generally large, typically 2×10^6 BCFP/ $m^2 h$ and no allowance was made for leakage of bacteria into the rooming apparatus. After allowing for differences between calves and between ages and their interactions, the residual sampling error (e.g. within calf variation) corresponded to a coefficient of

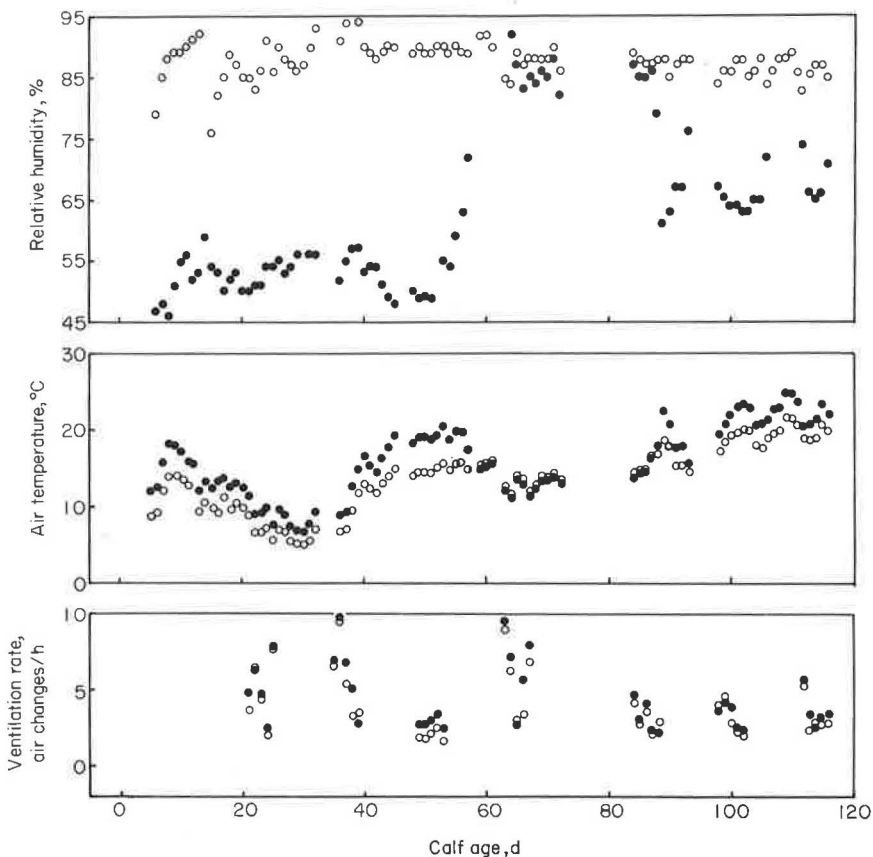


Fig. 3. Ventilation rate and mean relative humidity and air temperature in (○) the control and (●) dehumidified rooms during the experiment

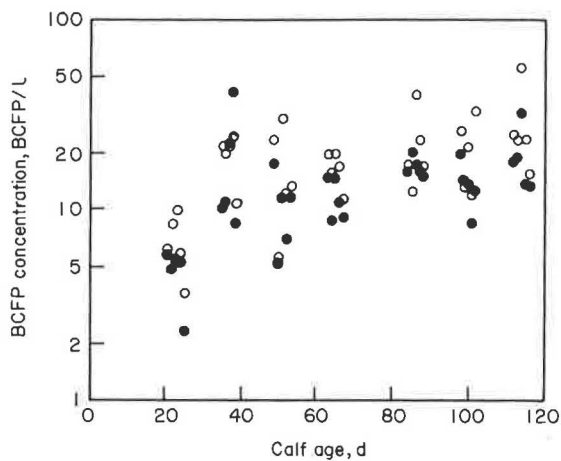
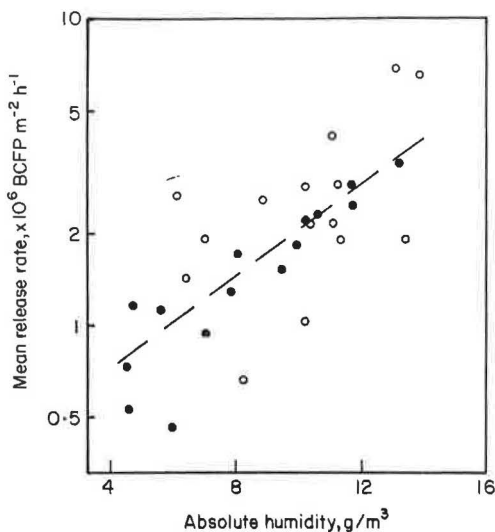


Fig. 4. Mean concentration of bacteria colony forming particles as a function of calf age in (○) the control and (●) dehumidified rooms



5. Mean flux of airborne bacteria vs absolute humidity for calves in (○) the control and (●) dehumidified rooms

tion of 2.8%. By comparison the concentration measurements had a within day (between days, between 2-week periods) C.V. of 6.2%. Measurements taken when the calf struggled or fell were discarded.

The daily balance of airborne bacteria for those days with simultaneous measurements of concentration and release flux is given in Table 1. Values of q_e were calculated from Eqn (3) and did not exceed 50 air changes/h. In deriving these estimates the calves were assumed to be the major source of bacteria and other possible contributions, such as the floor, were ignored. Contribution from the supply air was negligible.

5. Discussion

5.1. Physical and aerial environment

British calves can be housed in climatic buildings because they thrive in conditions covering a wide range of temperatures.¹⁶ In particular, average daily liveweight gain and total heat loss of calves is the same at air temperatures from 5 to 20°C but the proportion of heat lost by convection rises with temperature.¹⁷ Similarly, the direct effects of air humidity on calf performance are small and, in consequence, the recommended span is wide, 30–90% r.h.¹ In this experiment relative humidity never fell below 75% in the control room while air temperature was in the range 10–20°C, from which we may infer that the calves were neither heat nor cold stressed.

There have been many surveys of BCFP concentrations in animal houses: mean levels⁶ are about 100 BCFP/l compared with less than 30 BCFP/l in this experiment in which the cubic capacity was double that recommended for commercial farms.¹⁶ The rise in concentration with age is due not only to the higher flux from the animals but also to their larger surface area. The origins of airborne bacteria and dust in animal houses are the animals themselves and the stockman, bedding or litter, especially during bedding down and when disturbed, and the animal's movements.⁸ In our study the animals were the major source of bacteria and, when they were removed at the end of the trial, levels fell to one-sixth or less of those recorded during the animals' occupancy, in agreement with Goodrich *et al.*¹⁹

TABLE 1

Release and clearance rates and concentrations of airborne bacteria in two calf houses

Calf age, d	No. of calves	Mean calf weight, kg	Mean air temperature, °C	Mean relative humidity, %	Absolute humidity, g/m ³	Ventilation rate (q_v), air changes/h	Mean release flux, \log_{10} BCFP m ⁻² h ⁻¹	Total release, BCFP/h $\times 10^6$	Mean concentration, \log_{10} BCFP/m ³	Rate constant (q_c), equivalent air changes/h
25	9*	59	5.6	86	6.10	7.7	6.149	18.64	3.555	39.5
	9	59	7.7	54	4.38	7.8	5.717	6.90	3.361	19.5
36	9	73	6.7	91	6.85	9.5	6.277	28.27	4.295	3.5
	9	73	8.7	52	4.50	9.8	6.057	17.04	4.042	4.3
50	8	90	14.4	89	11.05	1.8	6.325	31.45	3.748	49.3
	8	90	18.9	49	7.95	2.7	6.226	25.04	3.721	40.6
64	8	107	11.6	84	8.70	6.2	6.402	41.39	4.197	17.7
	8	107	11.2	92	9.35	7.2	6.179	24.77	3.945	18.4
85	7	134	14.6	88	11.00	2.8	6.618	67.39	4.097	46.2
	8	134	14.4	88	10.55	3.1	6.360	42.52	4.295	16.5
99	7	155	18.2	86	13.40	4.4	6.278	33.59	4.129	18.3
	8	155	20.7	65	11.70	3.2	6.388	49.46	4.161	27.8
113	7	175	18.7	86	13.80	2.4	6.816	124.18	4.384	44.2
	8	175	20.6	65	11.65	3.4	6.455	61.81	4.278	26.2

*For each day the first and second rows of data correspond to the control and dehumidified rooms, respectively

5.2. Release rate of bacteria

an, the skin acts as a host for both aerobic²⁰ and anaerobic²¹ bacteria, which live in micro-colonies, each containing 10^2 – 10^5 cells.²² The bacteria are dispersed into the air on desquamated skin scales²² or squames, which are shed during normal replacement of the skin. Not all squames carry bacteria^{23, 24} and their median aerodynamic diameter is about $2 \mu\text{m}$ although their median minimum projected diameter is much larger.²⁴ The rate at which animals shed bacteria is highly variable and is faster in males than in females.²⁰ If measurement on the legs alone can be extrapolated to the whole body Mackintosh *et al.*²⁴ recorded from males ranging from 2×10^5 to 2×10^6 colony forming units $\text{m}^{-2} \text{h}^{-1}$. However, probably 80% of the output may come from the perineal region.²⁵ The bacterial fluxes observed in this study were very large, typically 2×10^6 BCFP $\text{m}^{-2} \text{h}^{-1}$ at the end of values reported for men. As stated earlier, our calves were not under severe stress and it seems likely that the relationship between R^* and ρ reflected either changes in cutaneous moisture loss with increasing temperature or age *per se*, or both. Certainly cornified skin is more easily detached from the bovine epidermis after swelling and water absorption and wetting and increased humidity²⁶ while the micro-climate beneath the hair coat and the supply of nutrients provide an attractive site for bacterial growth. The use of absolute humidity as an index of climatic stress is probably an oversimplification, and other variables, such as temperature and vapour pressure, or combinations of these, may be more appropriate. At air temperatures above 25°C there are large regional differences in sweating rate in young cattle,²⁷ which disappear at 15°C . Therefore, the extrapolation of measurements based on one region of the body to the whole animal may have caused uncertainties in the estimate of R . The total bacterial output to the room clearly depends on the number of calves and their activity. If animals are also on litter, disturbance of the bedding will disperse many bacteria into the air. Unfortunately, the contribution from this source are not available.

5.3. Survival of airborne bacteria

There have been many studies of the survival of airborne microbes which have been generated mainly in the laboratory.²⁸ In general, the lifetime of individual species of bacteria in an artificial atmosphere is shortened as air temperature rises, while the effects of relative humidity (at a constant temperature) depend on the species.¹³ However, when an aerosol contains more than one species, the case with one produced naturally, samples taken some distance from the source will include those species with long half-lives: only the survivors are collected. The effect of climate on a mixture of bacteria will be a function of their individual responses and therefore the relationship describing their combined response is unlikely to be simple. Furthermore, the survival of bacteria on skin squames will not necessarily be the same as that for bacteria suspended in

air. Our results show that q_e ranges between 4/h and 49/h compared with air change rates of 2–10 changes/h. The clearance mechanisms encompassed in the determination of q_e include both physical processes of sedimentation and impaction and the biological process of death. The former will be influenced by the airflow patterns in the building while the latter will be influenced by, amongst other things, climate. The relationship of q_e with both these determinates is complex. Although previous workers¹² have shown that the rates of physical decay can be determined from the clearance of physical tracers of similar aerodynamic properties,²⁹ the determination of a suitable index of climatic stress for a heterogeneous aerosol must await further work. Nevertheless, our demonstration that q_e is of magnitude comparable with air change rates determined in naturally-ventilated livestock buildings shows the relative importance of the different decay mechanisms, even though the factors which determine q_e are unknown.

5.4. Air hygiene and animal health

Although there has been much speculation regarding air hygiene and respiratory diseases of farm animals the evidence for the association is slight.^{6, 7} In the context of bacterial aerosols

two points must be made: first, the majority of airborne bacteria are non-pathogenic second, bacteria can provide a burden on the defences of the respiratory tract even after death.⁷ Pritchard⁵ suggests that impaired pulmonary clearance is a necessary cause of pneumonia. The impairment may result from "ciliary loss due to viruses or mycoplasma, bacterial endotoxin, noxious gases such as ammonia or to overburden of the mucociliary escalator by bacteria, fungi or dust".⁵ His later study³⁰ showed that filtration of air reduced the incidence and severity of respiratory disease in housed veal calves. The concentrations of both bacteria and viruses were lower in the filtered room and it is impossible to ascribe the benefit to either pollutant; indeed, both may have been implicated.

Mitchell's¹⁶ recommendations for the design of calf houses, in particular their ventilation, have been widely adopted by British farmers. His minimum ventilation rate of 6 air changes/h and a cubic capacity of 6 m³ per calf ensures that calf buildings are relatively spacious by traditional standards. One possible explanation for the success of his advice is that reducing the stocking density simply lowers the output of bacterial contamination. The importance of stocking density was also shown in one North American study³¹ in which raising the ventilation rate from 1 to 6 air changes/h had no effect on the clinical incidence of pneumonia ($\approx 80\%$). This was probably because of the low cubic capacity of 3.1 m³ per calf.

6. Conclusions

At present the concentration of airborne bacteria cannot be used as a criterion for ventilation rates, because the critical levels are unknown.⁷ Despite this lack, the concept of a microbial balance can aid the designer of animal houses in the understanding of the kinetics of bacterial aerosols. Recognition of the relative importance of pathways of clearance including ventilation and the strengths of the different sources will lead eventually to fundamentally sound designs for calf houses and ventilation systems.

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