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The survival of *Escherichia coli* in an aerosol at air temperatures of 15 and 30 °C and a range of humidities

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SUMMARY

The survival of *Escherichia coli* in an aerosol was studied at several temperatures and over a range of relative humidities using a Henderson apparatus.

Death occurred in two phases, the first lasting approximately 1 min; in the second the number of viable microorganisms declined exponentially. *E. coli* was robust and remained viable for many hours. Death was most rapid at low humidities ($< 50\%$ r.h.) at 15 and 30 °C, with half-lives of 14 and 3 min respectively. In humid conditions the half-lives were much longer, approximately 83 and 14 min respectively.

Based on this work, preliminary recommendations for the climate of livestock buildings can now be given to control the airborne spread of *E. coli*.

INTRODUCTION

Aerosolization is a traumatic process for most microorganisms. Their survival in the airborne state is determined by many factors; for example the method of culture, the composition of the suspending fluid, the mechanism of aerosolization and the climate into which they are launched (Anderson & Cox, 1967). Assessment of microbial survival is also governed by the collection technique employed, e.g. the nature of collection fluid and the type of sampler (see Strange & Cox, 1976). Amongst enteric bacteria in particular, the fate of *Escherichia coli* in an aerosol has been extensively studied over the past 30 years (Ferry, Brown & Damon, 1958; Webb, 1959; Cox, 1966*a*, *b*, 1970; Songer, 1967; Erhlich, Miller & Walker, 1970; Müller, Gröning & Hartmann, 1981). In general the survival of *E. coli* in the air is better at low than at high temperatures and in humid than in dry atmospheres. In other gases, e.g. nitrogen, the influence of humidity can be quite contrary and narrow zones of high sensitivity are also apparent, although the addition of a protecting agent to the suspending fluid, such as raffinose, can remove this sensitivity (Cox, 1966*a*).

The purpose of this study was to examine the effect of humidity on the survival of airborne *E. coli* at air temperatures of 15 and 30 °C. The work is part of a larger project looking at the aetiology of post-weaning diarrhoea in piglets, in particular the influence of temperature on the disease and the route of transmission of the infectious agent between animals. In this project 3-week-old piglets were challenged

orally with a pathogenic strain of *E. coli*. Some piglets showed symptoms of post-weaning diarrhoea at 15 °C, few at 20 °C and none at 30 °C. Furthermore, transmission and infection of unchallenged animals occurred by an airborne route at 15 °C, and this finding was confirmed when animals were exposed to an aerosol challenge of *E. coli* at the susceptible age (C. M. Wathes, B. G. Miller & F. J. Bourne, in preparation).

METHODS

Culture of organism and preparation of spray suspension

A nalidixic acid-resistant strain of *E. coli*, serotype 0149, was used. Cultures of the bacteria were grown overnight at 37 °C in a shaking water bath in the minimal medium of Davis & Mingioli (Cruickshank *et al.* 1973) containing 0·1 ml of 3·11 TBq $mmol^{-1}$ methyl [^{3}H]thymidine aqueous solution and 250 $\mu g\ ml^{-1}$ 2′-deoxyadenosine (Sigma Chemical Co.), which enhanced the uptake of thymidine (Boyce & Setlow, 1962). The overnight culture was centrifuged at 7400 *g* for 20 min and suspended in 10 ml phosphate-buffered saline to provide the spray suspension, with an approximate concentration of 10^9 colony-forming units (c.f.u.) per ml.

Aerosol apparatus

The bacterial aerosol was generated using a single-jet Collison nebulizer (May, 1973) operating in a mobile Henderson apparatus (Druett, 1969) and was stored in a 75 l rotating drum (Goldberg *et al.* 1958). Physical losses within the apparatus were measured by radiolabelling the bacteria (Anderson, 1966). The entire apparatus was placed in a temperature-controlled room. The aerosol samples were collected in raised, all-glass Porton impingers (May & Harper, 1957) containing 10 ml phosphate-buffered saline and 0·1 % antifoam RD emulsion (Dow Corning). The flow rate of the impingers was calibrated in triplicate and corrected to s.t.p. The mean ($\pm$S.E.) flow rate for the 24 samplers was $11{\cdot}0 \pm 0{\cdot}70$ l min^{-1}. Temperature and humidity were measured by wet-bulb hygrometry using a matched pair of mercury thermometers accurate to $\pm 0{\cdot}1$ °C.

Test procedure

The Henderson apparatus and rotating drum were conditioned at the required climate using a distilled water spray for at least 1 h before the start of each run. The spray was switched to the bacterial suspension and a sample of the aerosol from the spray tube was collected over 1 min. This was termed the 1 s sample, 1 s being the approximate time taken for the aerosol to travel from the nebulizer to the sampling point. The drum was then flushed with the bacterial aerosol for 5 min before it was sealed, and the age of the cloud was taken as zero at the end of this procedure. For the majority of runs, seven 1 min samples of this cloud were taken at cloud ages of 1 min and at other ages up to 2 h, the exact times depending on the expected half-life.

Bacterial assay

Serial dilutions of the impinger samples were made in one-quarter-strength Ringer's solution. Viable counts were assessed in sextuplicate using a modified

method of Miles & Misra. The counts of the physical tracer were measured in triplicate using a β-counter and were corrected for background counts and quenching losses.

Calculation of rate constant

The death of airborne bacteria can be separated into two phases, and the process in the second phase can be described by simple first-order kinetics (Webb, 1959). Within the drum, bacteria are subjected to both physical losses and biological death and their concentration, C, at cloud age t is given by $C = C_1 e^{-(q+p)\ (t-1)}$, where C_1 is the concentration at $t = 1$ min and q and p are rate constants for clearance by death and physical loss respectively. Similarly the rate of clearance of the tracer is given by $P = P_1 e^{-p(t-1)}$, where P is the count at time t and P_1 is the count at 1 min. Combining these two equations and taking logarithms gives a linear relationship between $\log (CP_1/C_1P)$ and $(t-1)$ with slope q, which can then be estimated by linear regression.

RESULTS

The survival of *E. coli* in an aerosol was studied at two air temperatures, nominally 15 and 30 °C, and over a range of relative humidities, and in total 26 series of measurements were taken. The mean average dry-bulb air temperatures (range) were 15·0 °C (13·2–16·9) and 31·2 °C (29·4–35·0) over a range of relative humidities from 32 to 87 % and 33 to 87 % respectively.

Fig. 1 shows one example of the series of measurements at a dry and wet bulb temperature of 15·2 °C and 11·0 °C respectively, equivalent to 60 % r.h., and is typical of the experiment. The concentration of viable bacteria, uncorrected for physical losses, was 1180 c.f.u./c.c. in the spray tube at 1 s, it fell rapidly to 100 c.f.u./c.c. after transit to the drum and storage for 1 min and decreased further to 44 c.f.u./c.c. after 100 min. After correction for physical losses there was a linear decline in the viable counts expressed as a percentage of the counts at 1 min. The rate constant for biological death, q, calculated from the regression, was $0{\cdot}53 \pm 0{\cdot}119$ h^{-1} ($F_{1,5} = 19$, $P < 0{\cdot}01$), equivalent to a half-life of 78 min.

Death during the first minute after aerolization was most rapid at mid-range humidities (45–65 % r.h.) and was fastest at 30 °C. For example, the proportionate viability of bacteria within the drum at a cloud age of 1 min relative to the viability in the spray tube at 1 s was approximately 0·38 and 0·065 at 15 and 30 °C respectively at 55 % r.h.

The rate constants for all 26 series are shown in Fig. 2 as a function of relative humidity, and all the linear regressions provided good statistical fits to the data ($P < 0{\cdot}05$). The overall pattern was similar at both temperatures, with the fastest death rate at low humidities. At 15 °C the rate constants were typically about 3 h^{-1} at 40 % r.h. and fell to about 0·5 h^{-1} at 80 % r.h. At 30 °C and the same humidities the corresponding rate constants were 13 and 2 h^{-1} respectively.

DISCUSSION

The loss of viability of airborne bacteria is the outcome of a number of complex phenomena and is usually characterized by a death rate which is initially fast and

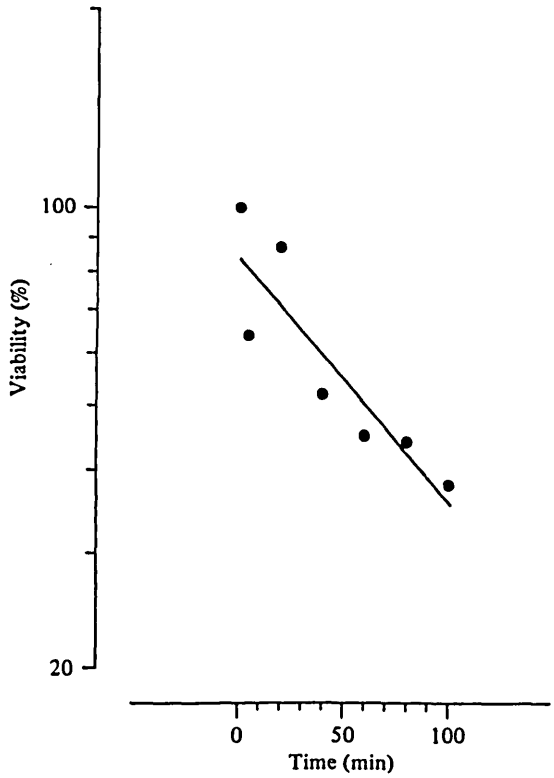

Fig. 1. Losses of aerosolized *Escherichia coli* with time during second stage of death expressed as organism viability relative to viability at a cloud age of 1 min (%).

subsequently diminishes progressively (Benbough, 1967). The first phase of death does not follow simple first-order kinetics and lasts approximately 1 min: in the second, the number of viable organisms declines exponentially (Ferry, Brown & Damon, 1958; Webb, 1959; Anderson, 1966; Benbough, 1967). Furthermore, despite sustaining substantial injuries during aerosolization, airborne microbes can still remain viable and may even recover fully given a suitable environment (Strange & Cox, 1976).

The factors which influence the survival of airborne bacteria include the strain of organism, the composition of culture and suspension fluid, conditions of growth and concentration procedures, and the atmosphere and climate into which the microbes are launched (see Strange & Cox, 1976). The method of sampling, in particular the composition of the sampling fluid and climate, and the tracer technique employed, also affect assessment of microbial survival (Anderson, 1966; Cox, 1966*b*; Strange *et al.* 1972).

Published studies on the survival of *E. coli* as a function of humidity have been conducted at temperatures different to those of this report and have, of course, used different strains. The work of Cox (1966 *et seq.*) was mainly conducted using nitrogen, in which survival is longer than or equal to that in air at low and high humidities respectively, and shows a zone of instability at high humidities for some spray and collecting fluids. Both Anderson (1966) and Webb (1959) have

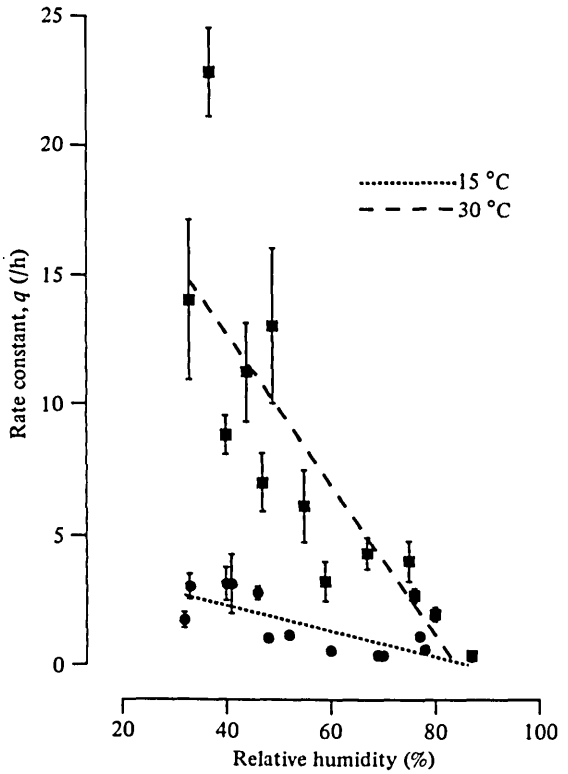

Fig. 2. Rate constants for second-stage death of aerosolized *Escherichia coli* vs relative humidity. Bars denote 1 S.E.

examined the effect of humidity over a wide range and agree with Ferry, Brown & Damon (1958) that death following aerosolization occurs in two stages, the first lasting about 20 s (Ferry, Brown & Damon), 10 s (Webb) or up to 5 min (Anderson). Our results show a marked effect of both temperature and humidity on the viability of *E. coli* in the first and second stages of survival, i.e. at cloud ages of 1 min and older. At air temperatures of 15 and 30 °C second-stage death rates at low relative humidities of 50 % and less were about six times faster than at high humidities. At the same r.h. the death rates of 30 °C were about four times faster than at 15 °C. Anderson's data, which are presented in the original in a form unsuited for re-examination, show a fourfold rise in q from 80 % to near saturation. Unfortunately, temperature control in the present study was not accurate enough to permit measurements at humidities above 90 % r.h. without condensation of the aerosol during storage. The overall agreement between other studies and these data as to the magnitude of q suggests that the effects of relative humidity are similar over a temperature range from 15 to 30 °C.

The mechanism(s) by which humidity influences survival were originally thought to be due to the rapid evaporation of free water from the aerosol droplet immediately post-aerosolization and to the diffusion of bound water from the bacterial cell in the quasi-equilibrium state of the second phase of death. However, Cox (1970) has shown that it is the stresses of rehydration and not dehydration

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which are responsible for the effects of humidity. In humid atmospheres RNA synthesis is affected, while in dry conditions flavin-linked enzymes are damaged by the action of oxygen (Benbough, 1967). However, the number of concurrent lethal processes make it difficult to identify any one mechanism as the cause of bacterial death in aerosol.

Distilled water and other neutral liquids, such as buffered saline, are probably the most common suspension fluids used in studies of microbial viability because they provide a convenient baseline for comparisons between organisms. However, the importance of the suspension fluid in determining survival has long been recognized (Strange & Cox, 1976); for example, simple compounds such as inositol and di- and trisaccharides are all protective (Webb, 1960). Naturally generated aerosols of *E. coli* are not formed from buffered saline but instead from a diarrhoeic slurry. Unfortunately few studies have been carried out involving 'natural' suspension fluids but it is likely that their effect will be protective due to their proteinaceous composition.

Our demonstration (C. M. Wathes, B. G. Miller & F. J. Bourne, in preparation) that susceptible pigs can suffer from post-weaning diarrhoea when challenged with an aerosol of this strain of *E. coli* and that transmission can occur by an airborne route clearly does not imply that this is the sole, or even the most important route. In our experience of caged piglets, infection and transmission in a group is apparently initiated by one pig which may have been infected by the aerial route and which then transmits the pathogen to the remainder, most probably via snout–anus contact. If this is the case, then there are several important consequences for the design and management of intensively housed piglets.

First, specifications of the climate within a piggery can now be established according to several criteria. The traditional criteria for climate control in an intensive piggery are based on the animal's thermal physiology, and the recommended climate is usually within the animal's thermoneutral zone (Bruce, 1981). Our data show that a warm, dry atmosphere (~ 30 °C, < 50 % r.h.) will favour the rapid death of airborne *E. coli* and help prevent the spread of post-weaning diarrhoea by the airborne route. The use of humidity and, by implication, temperature regulation as a prophylactic measure was suggested as long ago as 1948 by Dunklin & Puck, but until recently their suggestion has not been adopted in intensive livestock husbandry, probably because of a lack of basic data on the aetiology of airborne diseases (see Wathes, Jones & Webster, 1983). Secondly, our data show the relative importance of microbial death and ventilation as means of improving air hygiene in a piggery. Ventilation rates usually range from 2 to 20 air changes per hour and are thus of similar magnitude to death rates of airborne *E. coli*. Thirdly, solid pen divisions are preferable to open divisions, especially where airflow patterns within the building's encourage mixing between pens.

The simple, preventive measures described above are consistent with other good husbandry practices and should not necessitate major changes in the rearing of young piglets. Similar data on the survival properties of other agents of infectious diseases of housed farm animals could provide new recommendations for climate control based on health as well as production criteria.

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