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The effect of air ionization on the air-borne transmission of experimental Newcastle disease virus infections in chickens

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SUMMARY

The effect of artificial air-ionization on air-borne transmission of Newcastle disease virus (NDV) infection in chickens was studied in an isolated system consisting of two side-by-side cages with solid walls and a wire-gauze roof. During a 3-week observation period more than 90 % of the uninoculated indicator chickens, housed in one of the cages, contracted the virus shed to the air by the NDV-inoculated, diseased birds in the neighbouring cage. This air-borne transmission of NDV was completely prevented by increasing the ion concentration in the test room by a constant negative corona discharge above the wire-gauze roof. On the other hand, spreading of the infection within a group of chickens housed in a single cage was not affected by air ionization.

These and other results suggest that artificial air-ionization may protect animals from certain air-borne infections by interfering with microbial aerosol formation and/or by facilitating their decay.

INTRODUCTION

Particles suspended in air (aerosols) are known to be ionized carrying either negative or positive net charge. The pattern of aerosol ionization can be artificially modified by producing, for instance, with the aid of a corona discharge, large numbers of unipolar small ions in the air (Lehtimäki & Graeffe, 1976). The ionized aerosol particles have a tendency to move towards the opposite charge and consequently, in a closed space like a room, may be cleared from the air by trapping onto the walls or other charged surfaces. The rate of aerosol decay depends on several factors, including the net charge and the size of the particles. Studies on the effects of ionization on aerosols of biologically inert particles have revealed a non-linear relation between the size of the particle and the rate of clearing from a closed space (Lehtimäki & Graeffe, 1976). Many pathogenic viruses with proved or suspected air-borne route of transmission have diameters close to the most susceptible particle size in this regard (0·1–0·01 μm). Though it is unlikely that

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infectious virus aerosols mainly consist of single virus particles, it is possible that the degree of air ionization might influence the rate of decay of virus aerosols even more than that of bacterial aerosols (Mäkelä *et al.* 1979).

Being aware of the reported 'antimicrobial' effects of air ionization and of the lack of information about the possible mechanisms of these effects (Krueger & Reed, 1976), we have performed the present studies in order to find out whether air-borne transmission of experimental virus infections could be prevented by air ionization. Newcastle disease virus (NDV) infection in chicken flocks was used as the experimental model system.

MATERIAL AND METHODS

Test arrangements

The study was performed in an isolated inside unit consisting of a small anteroom for protective clothes and boots and an animal room of 10 m^2 floor area. Temperature of the room was kept between 15 and 20 °C and, during Expts. III–V, the relative humidity was adjusted to 75–80%. The chickens were kept in two cages located side by side and both having a floor of 100 × 80 cm. The walls of the cages were 36 cm high and were, like the floor, of impenetrable material. A wire-gauze roof with a mesh size of 2 × 2 cm was used on top of the cages. In Expts. III–V an electric fan was used to produce a constant air flow above the cages. Special care was taken to avoid accidental cross-contamination between the cages and the animal maintenance procedures were minimized during the experiments. After every single experiment the animal room and the cages were thoroughly disinfected chemically by 2% lye solution.

Chickens

Healthy 1- to 5-week-old chickens of the Leghorn strain were obtained from two poultry flocks with no recent history of serious infectious diseases. In each single experiment all the chickens were of the same age and from the same flock. Food and water were given *ad libitum*.

Virus

In the experiments we used a velogenic strain of Newcastle disease virus, isolated in Finland (Estola, 1974) and subsequently passaged 11–13 times in chicken embryos. The virus concentration in allantoic fluid harvests was 10^8–$10^{8\cdot7}$ EID50/ml or 10^8 TCID50/ml as titrated in chicken embryos or in cultures of chicken embryo kidney cells, respectively. Chickens inoculated with this virus developed symptoms of disease (dyspnoea, fatigue) generally within 2–3 days, and all but 1 out of 80 in these experiments died during the following 48 h.

Transmission experiments

At the beginning of each experiment a group of chickens (group A) was inoculated intratracheally with 0·3 ml of an NDV-containing solution and placed in one of the two side-by-side cages. The rest of the chickens (group B) were not inoculated but served as an indicator population for virus transmission. Group B was placed

either in the neighbouring cage ('air-borne transmission experiments') or in the same cage as the inoculated animals ('spreading experiments').

The chickens were observed for 21 days and death of the indicator chickens was taken as the criterion for virus transmission. The transmission was further demonstrated by isolating the virus from the carcasses in chicken embryos. All animals surviving through the whole observation period were killed and tested for the presence of circulating antibodies against NDV. Haemagglutination inhibition (HI) technique was used as described previously (Estola, 1974).

Attempts were also made to demonstrate that the inoculated chickens shed virus aerosols during successful transmission of NDV from group A to B. Samples of 100 l of air were drawn during 2 h from above the A cage through Millipore filters, type AAWPO3700. The filters were aseptically removed from the holders and immersed in sterile phosphate-buffered saline. Aliquots of the eluate were inoculated into chick embryos. No growth of NDV was detected, suggesting that the sensitivity of the procedure was not sufficient for detecting NDV aerosols.

Ionization of the air

Artificial air-ionization was brought about by an apparatus consisting of a set of four free corona needles, −5 kV each (Ilmasti Oy, Helsinki), hanging above the wire-gauze roof of the group-A cage. The needle tips were stretched out to cover the cage and were each at a distance of 56 cm from the floor of the cage. The apparatus, when used, was switched on at the time of inoculation of group A and kept on throughout the whole observation period. A single 5 kV corona needle of this type has been shown to generate an ion current of 1·5 pA in a closed space. This was found to result in aerosol decay rates with half-life of 7, 117 and 180 min for particles with a diameter of 0·01, 0·1 and 1 μm respectively (Lehtimäki & Graeffe, 1976).

RESULTS

Air-borne transmission

Chickens inoculated with NDV shed infectious aerosols into the air as shown by successful transmission of the virus to the indicator chickens in Expt. I. Six out of eight chickens of group B contracted the disease and died during the observation period (Table 1). In contrast, no virus transmission from group A to group B was observed in Expt. II, where the corona discharge apparatus was kept on so as to increase the ion concentration of the air (Table 1). The survival time of the inoculated chickens (group A) was also slightly prolonged as compared with Expt. I. The latter phenomenon was, however, not seen in later experiments, suggesting that air ionization, under the conditions used, did not modify the pathogenesis of intratracheally inoculated NDV. Chickens surviving through Expt. I and II were tested for the presence of HI antibodies against NDV. All sera were negative at a dilution of 1/5.

In our second pair of experiments (not tabulated) all chickens in group B survived even without ionization in spite of the normal rapid death of the inoculated chickens. Absence of measurable HI antibodies in the sera of group-B

Table 1. *Inhibition by air ionization of air-borne transmission of Newcastle disease virus in chicken groups*

Ion generator	Chicken group	Deaths per consecutive day after inoculation: Day... 2	4	6	8	10	12	14	16	18	20	Total
		Experiment I										
	(A) Inoculated	– –	8									8/8
Off	(B) Indicator	– –	– – –	1	– 1	1	1 –	2				6/8
		Experiment II										
On	(A) Inoculated	– –	1	3 –	2							6/6
	(B) Indicator	– –	– – –	– –	– –	– –	– –	– –	– –	– –	– –	0/6

A group of chickens was inoculated intratracheally with $0{\cdot}3 \times 10^{8{\cdot}7}$ EID_{50} of Newcastle disease virus while the other group of chickens in the neighbouring cage was left uninoculated (indicator group). Spreading of the virus from group A to B was scored by recording the date of death of the indicator chickens. After the first experiment under normal conditions (I) the room and cages were decontaminated, and a similar transmission experiment was performed with the ion-generating apparatus on (II, see Methods).

–, No deaths recorded.

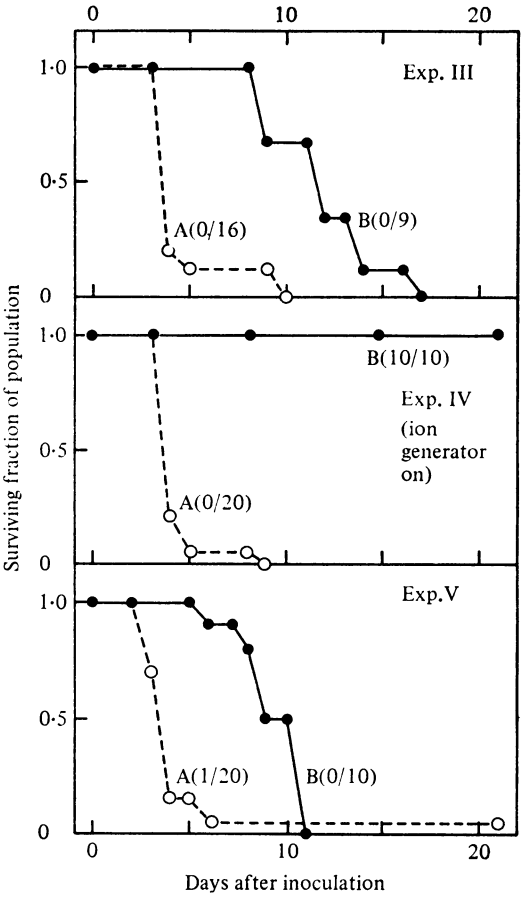

Fig. 1. Effect of air ionization on air-borne transmission of Newcastle disease in chickens. For experimental design see Table 1 and the text. Three successive experiments with (IV) or without (III, V) ionization. ○---○, inoculated chickens (group A); ●——●, indicator chickens (group B), housed in the neighbouring cage. Figures in parentheses show the number of chickens surviving for 21 days over total number of animals in the group.

chickens suggested that neither immunity nor subclinical infection was likely to be the reason for the survival. Two possibilities were considered and, in later experiments, measures were taken to eliminate the assumed reasons for poor transmission.

First, the high dose of NDV inoculated to group A chickens might have killed the animals too rapidly – serial dilutions (10^{-2} to 10^{-6}) of the virus were used in subsequent experiments so as to prolong the time the virus was shed into the air. However, dilution of the inoculum virus did not significantly prolong the time of survival of group A (data not shown). Secondly, the change of the climate towards lower relative humidity had taken place at the time of this 'failed' pair of experiments. Thus, later on, the relative humidity of the test room was controlled and kept between 75–80 %, and a fan was used to maintain an air flow above the

Table 2. *Spreading of experimental Newcastle disease virus from inoculated chickens to an indicator population housed in the same cage allowing physical contacts*

Ion generator	Chicken group	Deaths per consecutive days after inoculation: Day... 2	4	6	8	10	12	14	16	18	20	Total
		Experiment I										
Off	(A) Inoculated	– – 5										5/5
	(B) Indicator	– – – 1 2 – 1 1										5/5
		Experiment II										
On	(A) Inoculated	– 4 1										5/5
	(B) Indicator	– – – 4 – 1										5/5

For experimental details see Table I.

cages in the direction from group A to B. Transmission of the virus from group A to group B in the neighbouring cage was very efficient in subsequent control experiments (III and V in Fig. 1), possibly because of the latter two measures. These measures did not alter the protective effect of air ionizations as in Expt. IV, where the ion generator was used, no transmission of infection could be documented (Fig. 1).

When all five tabulated air-borne transmission experiments are combined we can see that all 16 indicator chickens (100%), which were exposed to air-borne NDV infection under the artificial ionization, survived (B groups in Expts. II and IV), while in the absence of ionization 25 out of 27 chickens contracted the infection and died (B groups in Expts. I, III and V) and only 2 (7%) survived.

Spreading of infection inside the cage

When the inoculated chickens (group A) and the indicator animals (group B) were placed in a single cage, allowing direct physical contact between the two populations, all the indicator chickens contracted the disease and died whether the ion generator, placed above the cage, was on or off (Table 2).

DISCUSSION

Many important infectious diseases of man and animals are transmitted from host to host by viral, bacterial or fungal aerosols. Formation of infective aerosols is influenced by several factors like the site of the infection, which, among other things, may provide the microbes with carrier particles such as fluid droplets derived from the mucosa of the respiratory tract or pieces of scaled epidermis (Noble & Somerville, 1974). The rate of decay of infectious aerosols is in turn determined by two groups of factors – those affecting the physical stability of the aerosols and those influencing the rate of biological inactivation of the microbes.

Measures presently available to control the spreading of air-borne infections – such as laminar (filtered) air flow systems – are readily applicable to small isolation units but are either ineffective or far too expensive and complicated to be used on a large scale outside the laboratory. An alternative approach to reduce the concentration of infectious aerosol particles might be to produce into the air large amounts of free small ions which would subsequently charge the aerosol particles and thus facilitate their decay.

Recent clinical studies by our group have shown that shedding of bacteria (*Staph. aureus*) into the air from open infected skin burns is effectively inhibited by a continuous corona discharge in the ward room (Mäkelä *et al.* 1979). The present results extend those findings and suggest that transmission of certain air-borne virus infections could also be limited with the aid of ion generators.

Despite the large amount of natural virus infections that is transmitted through the air, there are not many reliable experimental models for studying the air-borne transmission. Our original plan was to use, instead of NDV, the avian infectious bronchitis virus as the model but preliminary experiments revealed that the infection was not transmitted by air under the conditions used. Hence, we found it justified to infect the chickens with the velogenic strain of Newcastle disease

virus. Even with this highly contagious virus the transmission had to be secured by special test arrangements as described above.

Two out of 8 chickens of the indicator (B) group in Expt. I survived through the whole observation period. They did not have HI antibodies against NDV, suggesting that specific immunity was not the basis for the survival (Finland has since 1973 been totally free of Newcastle disease and vaccinations against it are not allowed). The most plausible explanation for the escape from infection is that the air-borne transmission of the virus from the inoculated chickens succeeded only with some of the indicator animals, and that the subsequent spread of the infection within group B was too slow to kill all the chickens during the observation period.

Although it is relatively easy to quantitatively measure the effects of artificial air ionization on the decay of aerosols in a dead space (Lehtimäki & Graeffe, 1976), our attempts to quantitate the influence of corona discharge on air ion concentration in our isolation unit failed, probably because of highly variable total particle content in the air. However, there is no doubt that the ion generator used in these studies effectively produced large amounts of negative ions in the air (Lehtimäki & Graeffe, 1976).

We assume that air ionization in our experimental system reduced the concentration of infective NDV aerosols in the test room. Direct evidence to support this idea could not be obtained, probably because of the low sensitivity of the sampling system used. This remains, however, the most plausible explanation for the observed protection from air-borne NDV infection as shedding of the infectious virus by the inoculated chickens was not, at least not drastically, affected by air ionization (Table 2). Furthermore, no evidence was obtained to suggest that air ionization could have increased the resistance of chickens to NDV. The latter alternative cannot, however, be completely excluded by these studies as a theoretical possibility, because relatively large inocula of the virus were used and, secondly, because the wire gauze used on top of the cages is likely to modify the effects of an external ion generator inside the cages. The modifying effect of the wire gauze should also be taken in account in interpreting our 'negative' results on the spreading of NDV within a single cage (Table 2).

Our experimental system was not designed to distinguish between the effects of ionization on the formation of infective aerosols and those on the stability of the aerosols. If the ion current generated by corona discharge can reach the site of aerosol formation, it is possible that the shed particles are rapidly charged and trapped in the immediate vicinity of the site of formation (Mäkelä *et al.* 1979), i.e. in our case on the respiratory mucosa of the inoculated chickens. Alternatively, charging of the virus when already suspended in air would facilitate trapping of the aerosols on the walls and floor of the cages. Which of these alternatives is more important in this experimental model, remains open to speculations.

Air-borne virus infections cause severe medical, veterinary and economic problems all over the world. We have described in this paper that increasing the ion concentration of the air by a corona discharge will efficiently protect chickens from air-born transmission of lethal Newcastle disease virus infection. Although our experimental conditions are highly different from the conditions in hospital

wards or, say, poultry farms, these results call for field trials testing their applicability in the practical control of air-borne infections.

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REFERENCES

ESTOLA, T. (1974). Isolation of a Finnish Newcastle disease virus with an exceptionally high thermostability. *Avian Diseases* **18**, 274–7.

KRUEGER, A. P. & REED, E. J. (1976). Biological impact of small air ions. *Science, New York* **193**, 1209–13.

LEHTIMÄKI, M. & GRAEFFE, G. (1976). The effect of the ionization of air on aerosols in closed spaces. *Proceedings of the 3rd International Symposium on Contamination Control, Copenhagen* **2**, 370–82.

MÄKELÄ, P., OJAJÄRVI, J., LEHTIMÄKI, M. & GRAEFFE, G. (1979). Studies on the effects of ionization on bacterial aerosols in a burns-and-plastic-surgery unit. *Journal of Hygiene* **83**, 199.

NOBLE, W. C. & SOMERVILLE, D. A. (1974). *Microbiology of Human Skin*. London: Saunders.