*J. Hyg. Camb.* (1961), **59**, 285
*Printed in Great Britain*



# Newcastle disease as a model for studies of experimental epidemiology

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(*Received* 3 *February* 1961)

## INTRODUCTION

Much remains to be learnt about the epidemiology of respiratory infections of man: some remarkable aspects of the 1957 pandemic of Asian influenza have made that very evident. One imagines that useful light might well be shed by studies on experimental epidemiology, a field much neglected since the classical work of Topley (1919) and his colleagues and of Webster (1924). A useful model for work on respiratory virus infections has been hard to find, but it seems that some strains of Newcastle disease virus (NDV) may provide what is wanted.

## MATERIALS AND METHODS

### *Virus strain*

The neurotropic Boney strain of NDV, kindly given us by Dr F. S. Markham of Lederle laboratories, was found to be highly contagious and lethal for young chicks. Virus was stored as infected allantoic fluid at $-65°$ C.; chicks from an uninfected flock, usually 1–5 days old, received 0·1 ml. of a 1 in 10 dilution of this, given as 1 drop into each conjunctiva and on to each nostril. Since infected chicks were never seen to recover, they were ordinarily killed as soon as advanced symptoms developed and this is recorded as 'day of death'. Chicks which failed to show symptoms were invariably found to be fully susceptible on later challenge. With this extremely virulent strain, therefore, there seemed to be no subclinical immunizing infection.

### *Contact experiments*

For contact experiments, chicks were housed in Topley cages, metal cages 18 in. × 18 in. × 18 in., having a glass window at the side to admit light and a lid, on top, of perforated metal. The 'litter' on the floor consisted of sawdust. No evidence was encountered suggesting that spread occurred from one Topley cage to another.

In the latter half of 1959 our work was temporarily held up. Contact experiments, hitherto satisfactory, began to give negative results. Sera from birds in the flock providing our chicks were accordingly examined and found by a neutralization test in tissue culture, though not by haemagglutinin-inhibition, to neutralize the virus. This result indicated that an undetected infection with an avirulent strain of NDV had spread to the flock; on changing to another flock, we obtained satisfactory results once more.

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*Recovery of virus from exposed plates*

Exposure of plates has proved a valuable technique for demonstration of bacteria in samples of air. We have found that a similar technique can be used for Newcastle disease virus. Into each of a series of Petri dishes 9 cm. in diameter was pipetted 2 ml. medium containing 1% horse serum, 9% Hedley-Wright broth, pH 7·6, 90% normal saline, with antibiotics as described below. The Petri dishes were placed on the floors of open and closed cages, their centres being 10 and 25 cm. from the wire barriers retaining infected chicks. The covers of the dishes were removed for various periods between 30 min. and 4 hr., care being taken that the whole area of the dishes remained moist.

After exposure, distilled water was added to make up the original volume of fluid in the Petri dishes and thereby compensate for any salt concentration caused by evaporation. The fluid was then placed on monolayers of chick embryo cells prepared and grown with *tris* (hydroxymethyl) aminomethane buffer as described by Porterfield & Allison (1960). Adsorption of virus by the cells was allowed to take place for 2 hr. at 37°, after which the fluid was removed and the cells overlaid with *tris*-buffered agar containing higher concentrations of antibiotics than usual (400 units penicillin, 400 $\mu$g. streptomycin and 200 units Nystatin-Squibb—per ml.). These concentrations of antibiotics did not reduce the plaque count with the Boney strain of Newcastle disease virus, as compared with plates without antibiotics, and they were usually able to suppress growth of contaminant bacteria and fungi. However, in each group of plates exposed some were contaminated, and occasionally all. In most experiments enough plates were free of contamination to establish unambiguously that plaques were present in some groups of plates but not in others (Tables 4 and 5). The plaques were counted after 3 days' incubation at 37° C. They varied somewhat in size and had the punched-out appearance with well-defined edges characteristic of NDV. Virus recovered from some of the plaques was propagated and shown to be NDV by haemagglutination-inhibition and neutralization.

It should be recognized that the number of plaques counted in these experiments does not represent the total number of infectious particles reaching the plates. Only a fraction of the virus in the 2 ml. inoculum will be adsorbed by the cells and some virus will be lost through thermal inactivation. Nevertheless, the number of plaques recovered gives a relative indication of the amount of virus reaching a defined target under various conditions.

## RESULTS

*Preliminary experiments*

In a number of early experiments three chicks were infected as described above and three normal contacts placed in the same cage 24 hr. later. Under optimal conditions such contacts all became infected and died within 14 days, most often in a week or less. Their symptoms were characteristic: paralysis of wings and legs was usually preceded or accompanied by rhythmical jerking movements especially of the head and neck; chicks were prostrate for 12–24 hr. before death. There was

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thus no difficulty in distinguishing between specific and non-specific deaths. Chicks infected by contact would in turn infect fresh contacts and so on through at least six lots of chicks in series. In assessing results it was necessary to take account of the possibility that from the originally inoculated (donor) birds only one contact chick contracted infection and that the virus was then passed on to the other normals in a second cycle. Since death after contact infection hardly ever occurred in less than 3 days after exposure, we felt safe in reckoning as infected by the original contact all birds dying 3 days or less after the first chick in a cage had died. Birds dying later were excluded from our calculations as 'uninterpretable'.

*Method of transmission: barrier experiments*

In hope of determining whether transmission of virus was by the air or otherwise, normal chicks were exposed to infected ones in divided cages. The cages were divided in one or other way as shown in Table 1. Vertical wire grids of half-inch mesh were either single or double, the two grids in the latter case being separated by 2 in., 6 in., 9 in., or 12 in. Where solid metal or glass barriers were used, measurements given are from ground level, the barrier being continued to the top of the cage as a wire grid. Where we used 6 in. of separation or more, a large type of cage was used—the 'tanks' described below. Exposure across these barriers was for at least 24 hr.; in practice this period gave as many takes as did longer exposure.

Table 1. *Infection of chicks across a barrier*

| Barrier ... | + | − |
|---|---|---|
| Single wire | 51 | 27 |
| Double wire (2 in. separation) | 12 | 1 |
| Double wire (6 in. separation) | 2 | 6 |
| Double wire (9 in. separation) | 0 | 10 |
| Double wire (12 in. separation) | 0 | 4 |
| Metal 5 in. high | 0 | 12 |
| Metal 10 in. high | 2 | 7 |
| Glass 5 in. high | 10 | 6 |
| Glass 8 in. high | 0 | 15 |

+ and − signs in Tables 1–4 indicated numbers of chicks dying (+) or surviving (−).

Table 1 shows that transmission through a single barrier, or two close together, took place very readily; with 6 in. of separation, it was less effective, while 9 in. prevented it.

The efficacy of the 5 in. and 10 in. metal barriers and the 8 in. glass barrier was surprising. The results indicate that infection is being carried either by rather large particles which settle quickly or by smaller ones which cannot survive in the air long enough to travel even for a few inches. The failure of the 5 in. glass barrier to stop cross-infection is readily explained by experiments reported later under 'Social factors'. The particles in question seem not to consist of redispersed infected litter, as the next paragraph shows.



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*Role of infected litter*

In contrast to influenza, NDV is excreted in the faeces and can contaminate the litter. We readily confirmed the work of other workers by recovering virus from sawdust mixed with faeces from a cage which had contained infected chicks. A suspension diluted $10^4$ times was still active. To determine the role of litter we removed from their cages chicks at various stages of illness; in some instances chicks had recently died in these cages. Food-pellets and drinking water were also removed and normal chicks allowed to run on the infected litter for a period of several days. To our surprise five experiments involving thirty normal chicks afforded no single instance of transmission of infection by this means. In one of these tests the food and drinking water were not removed; yet cross-infection did not occur. In one further test infected drinking water did transmit virus. Contamination of drinking water is, of course, notoriously a way of transferring this infection and this route has been deliberately used as a means of spreading an avirulent, immunizing strain (Winterfield & Seadale, 1956).

Failure of contaminated litter to infect may be explained in terms of Kohn's (1955) finding that the alimentary route is some hundreds of times less effective than the respiratory.

Table 2. *Time of maximal infectivity*

| | | In same cage | | | Across wire barrier | | |
|---|---|---|---|---|---|---|---|
| Day of exposure | ... | 2–3* | 3–4 | 4–5 | 2–3 | 3–4 | 4–5 |
| Transmission + | | 1 | 4 | 3 | 0 | 3 | 2 |
| Transmission − | | 5 | 0 | 3 | 6 | 3 | 3 |

* Day of inoculation reckoned as day 1.

*Time of maximal infectivity*

The results of several trials suggested that chicks were most infectious late in the course of the infection, particularly in its last 36 hr.

In Table 2 are summarized the results of four experiments—two where chicks were together in one cage, two in which infection across a barrier was concerned. A few cross-infections occurred when exposure was made within a few hours of inoculation; possibly the original inoculum was in part ejected. Attention was therefore concentrated on successive 24 hr. periods beginning 24 hr. after inoculation; three contact chicks were exposed to two or three 'donors', then removed to a clean cage. Numbers of chicks infected are recorded. Donor chicks died on the fourth or fifth day. It is clear that infection did not take place to any important extent before the period from day 3 to day 4, by which time chicks had usually developed advanced symptoms.

*Duration of contact*

Table 3 (right-hand side) shows the results of experiments with three (occasionally two) donor chicks with exposure in divided and undivided cages during the optimal period of infectivity.

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*Number of donors*

The results already described have been obtained when there were two or three donor chicks. When only one infected donor was used, the cross-infection rate was much the same (cf. Table 3). We have excluded from the table experiments in which exposure did not cover the optimal period (last 36 hr. of life of donors). It will be seen that with 24–48 hr. exposure there was a high rate of cross-infection whatever the number of donors, while with periods of less than 24 hr., successes were much less frequent in any group.

Table 3. *Effect of varying the number of donors and of period exposure*

| Hours of exposure | Barrier (single wire) | Takes with 1 donor | | Takes with 2 or 3 donors | |
|---|---|---|---|---|---|
| | | + | 0 | + | 0 |
| 1 | 0 | . | . | 0 | 3 |
| 2 | 0 | . | . | 0 | 9 |
| 4 | 0 | 3 | 3 | 6 | 27 |
| 8 | 0 | 0 | 9 | 3 | 3 |
| 13 | + | 3 | 15 | . | . |
| 24 | 0 | 5 | 6 | 20 | 3 |
| 24 | + | 4 | 3 | 45 | 21 |
| 48 | 0 | 8 | 0 | . | . |
| 48 | + | . | . | 22 | 5 |

*Ocular route of infection*

If infection was mediated by settling of coarse particles, it could be argued that the conjunctiva offered a larger target for these than did the nostrils. Accordingly a number of chicks were fitted with 'spectacles', pieces of transparent plastic fixed so as to cover the eyes, but not in an air-tight manner, and leaving the nostrils exposed. These were contacted with infected birds, some in divided, others in undivided cages. Some birds managed to scratch their spectacles off or to perforate them: of four in which the spectacles remained intact during 24 hr. of contact, all came down as quickly as did the controls, or even faster. This result thus failed to support the idea that ocular infection was of much more importance than the nasal.

*Social factors*

A surprising result was encountered in the course of tests designed to measure the distance over which infection would spread. Instead of Topley cages we used 'tanks', metal boxes 1 ft. 6 in. × 4 ft. and 1 ft. 6 in. high and having no roof. These were divided into three compartments by means of wire grids; infected (donor) chicks were placed in an end compartment and contacts in the others. In contrast to the experiments in closed Topley cages, almost no transmission occurred in the tanks, even to chicks in the immediately adjacent compartment. The explanation remained obscure until attention was paid to the behaviour of the chicks. These are very social animals and collect as near as possible to other chicks even when there is a wire barrier between. Chicks in the compartment B next to the infected ones



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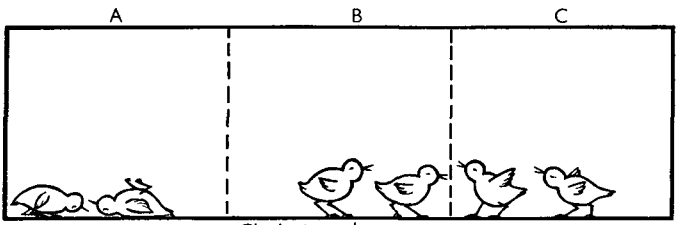
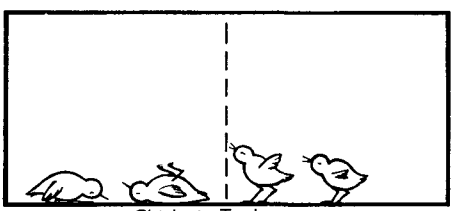
(in A) (see Fig. 1) ceased to take any interest in these as soon as they became ill but rather crowded up against the normals in the next cage (C), right away from the infected birds.

As already shown, chicks are particularly infectious in the 36 hr. or so before death, just at the time when they ceased to be socially attractive to the normal contacts. In the Topley cages, on the other hand, the contacts had no tendency to remain in the part of the cage remote from the infected birds. Experiment soon showed that this explanation was correct. When the tank experiment was rearranged so that infected birds were in compartment B and the contacts in A and C, transmission occurred to three of six exposed birds (as against one of twenty-one




Chicks in tank



Chicks in Topley cage

Fig. 1

before positions were reversed). In another experiment normal chicks were added to cage B containing the infected donors so as to serve as attractive 'social contacts' for the normals in A and C: five out of six of the normals in A and C went down with the disease within 4 days. In the experiments reported in Table 1 carried out in the tanks to permit separation over 6 in., 9 in. and 12 in. distances, care was taken, either by provision of 'social contacts' or otherwise, to avoid misinterpretation of results through the operation of the factors just discussed. Recognition of this social factor makes it easy to account for the spread of infection across a 5 in. solid glass barrier but not over a similar metal partition: chicks collected together only when they could readily see each other.

*Effect of covers on cages*

The difference between results in closed Topley cages and in open tanks was only partly explained by elucidation of the social factor. Table 4 shows that there was a striking difference between the outcome in open-topped tanks and tanks with perforated zinc covers, and a similar but smaller difference in the case of open and

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covered Topley cages. The tank experiments were carried out in the original way with donors in A and contacts in B and C.

It seemed possible that in the dim light beneath the perforated metal tops, the behaviour of the chicks would change and that they would cease to aggregate near the other normals and away from the sick birds. Those tops were therefore replaced by glass, leaving just enough space for ventilation: cross-infections still took place (8/24 positive). There is thus no evidence that intensity of visible light affected the issue either by changing the behaviour of the chicks or by a viricidal effect on particles in the air. The explanation of the differences between the figures in the 3rd and 4th horizontal columns of Table 4 remains obscure; presence or absence of a perforated cover can hardly be expected to affect ventilation at the bottom of a deep cage in such a manner as to prevent the coarse particles, which seem to be concerned, from reaching their target.

Table 4. *Effect of covers on cages*

| Type of cage | Transmission | | % positive |
|---|---|---|---|
| | Positive | Negative | |
| Closed Topley | 51 | 23 | 69 |
| Open Topley | 16 | 27 | 37 |
| Closed tank | 17 | 14 | 55 |
| Open tank | 1 | 20 | 4·5 |

Humidity readings were not very different in the four types of cage, varying only from 68 % in closed Topleys to 59 % relative humidity in open tanks. Some viruses however, are known to be labile in a narrow critical zone of relative humidity (Hemmes, Winkler & Kool, 1960); the role of humidity therefore needs further investigation.

*Virus recovery in exposed plates*

Dishes with broth were exposed in Topley cages and tanks for various periods at two times after inoculation of the donor chicks and at distances of 10 and 25 cm. from the barrier holding the infected chicks. The results of such experiments are summarized in Tables 5 and 6.

Experiments with Topley cages with closed tops are summarized in Table 5. Certain conclusions can be tentatively drawn from these results. First, more virus is recovered during the last 12 hr. of life of infected birds than earlier. The plates marked 'subterminal' were placed alongside chickens that were paralysed and showing forcible, convulsive, respiratory movements. In these more virus was recovered in 30 min. periods than was found in other plates exposed at other times for longer periods. Secondly, increasing the distance between the exposed plates and infected chickens greatly reduces virus recovery. Thirdly, the figures obtained are consistent with the interpretation that infection is mediated by droplets carrying several plaque-forming units of virus, and that increasing the duration of exposure under comparable conditions merely increases the probability that one such particle, containing viable virus, will reach the target.

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In other experiments the amount of virus recoverable in closed Topley cages and open 'tanks' was compared. Representative results summarized in Table 6 suggested that less virus was recoverable in the open tanks, from which it would appear that conditions in the latter are less favourable for survival of virus in infected droplets.

Table 5. *Plaques from plates exposed in the vicinity of infected chicks in Topley cages with closed tops*

| Time of exposure | Duration of exposure (min.) | Distance from carrier: 10 cm. | Distance from carrier: 25 cm. |
|---|---|---|---|
| 2nd day after infection | 60 | 0, 0, 0 | 0, 0 |
| | 120 | 0, 6, 0 | 0, 0, 0 |
| 3rd day after infection | 30 | 22, 0, 0 | 0, 0, 0 |
| | 60 | 14, 6, 0 | 0, 0, 2 |
| | 240 | 8, 20 | 0, 0 |
| Subterminal | 30 | 18, 26 | 0, 0 |

Numbers separated by commas represent plaque counts in replicate plates.

Table 6. *Plaques from plates exposed in the vicinity of infected chicks in closed Topley cages and open tanks*

| Time of exposure | Duration of exposure (min.) | Closed Topley: Distance from carrier: 10 cm. | Closed Topley: Distance from carrier: 25 cm. | Open tank: Distance from carrier: 10 cm. | Open tank: Distance from carrier: 25 cm. |
|---|---|---|---|---|---|
| 3rd day, Expt. 6 | 60 | 8, 5, 0, 0 | 0, 4, 0 | 0, 2, 0, 0 | 0, 0, 0 |
| 3rd day, Expt. 7 | 60 | 14, 6, 0, 0 | 0, 0, 0, 0 | 0, 0, 0, 0 | 0, 0, 0 |

## DISCUSSION

Conclusions based upon this work may well be applicable only in so far as the Boney strain of NDV and chicks of a particular breed and age are concerned. With this reservation, our experiments indicate that cross-infection in Newcastle disease is mainly air-borne. True, contaminated drinking water can also infect, but transmission readily occurs where this vehicle is excluded. Infected litter failed, in our experience, to transmit the disease. Results of experiments involving barriers may be explained in either of two ways. The effective particles may be coarse so that they cannot be wafted very far. Alternatively the suspended particles are small but very labile. On either explanation the effect of duration of exposure indicates that a rather infrequent but highly effective event may transfer infection to the normal chicks. Longer exposure would simply increase the chances of this occurrence—one can hardly imagine a gradual build-up to a critical concentration in the air either of coarse virus-carrying particles or of very labile small particles. What the infrequent effective event could be remains obscure: we have never seen

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the infected birds cough or sneeze; they have, however, in the terminal stages, a rather forcible spasmodic respiration.

The results with NDV have a possible bearing on the epidemiology of respiratory infections of man. It is remarkable that close contact of people in crowded trains seems relatively ineffective in transferring infection, possibly because contact of donor and recipient is relatively short. On the other hand infection spreads much more readily in the home, on board ship and in service messes, where the parties to the potential virus transfer are together for longer periods. The infrequent but effective event may here be a sneeze, a cough or forcible saliva-scattering speech.

We obtained divergent results when using cages of different types; these divergences proved to be explained, in part, by an effect on the social behaviour of the chicks, leading to congregation near other normals and away from sick birds. Studies on the epidemiology of human respiratory infections have tended to focus on the viability of the infective agents; one is tempted to speculate whether it might not be as profitable to pay attention to the social behaviour of the virus' potential victims.

SUMMARY

Observations are reported on the mechanism of transmission of a neurotropic strain of Newcastle disease virus from infected to normal baby chicks. Infection appeared to be mediated mainly by large or very labile air-borne particles. Other factors examined were the time of maximum infectivity of chicks, the effects of duration of contact, of the social habits of the chicks and of the type of cage used to house them.

Results obtained by contact experiments were supported by tests in which settling virus particles were collected in Petri dishes placed at various distances from infected chicks.

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