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The evidence for the airborne spread of Newcastle disease

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SUMMARY

Newcastle disease virus has been shown to survive when airborne in small particles, both in the laboratory and in the open air. Field outbreaks have been studied and viable virus has been recovered from the open air short distances downwind of infected premises. Vaccination of birds leads to a great reduction in the amount of virus liberated into the air.

INTRODUCTION

Newcastle disease is a highly infective and contagious disease of poultry. It causes outbreaks from time to time throughout the world and losses from the disease in Great Britain alone can amount to £20,000,000 annually.

Newcastle disease virus (NDV) has been classified in numerous ways (National Research Council, 1971), one of which designates the virus as pneumotropic, neurotropic or viscerotropic. These terms are subjectively derived and are based mainly upon the symptoms and lesions observed after natural or laboratory infections. The Asiatic form of the disease has been associated with visceral lesions in infected birds, rapid and very high mortality, and a low tendency to spread from farm to farm. Some laboratory strains such as Herts '33 are associated with symptoms in which paralysis and other neurological derangements predominate. Many British field-strains before 1970 were from outbreaks which showed a mixture of nervous and respiratory symptoms.

Field-studies of the present epizootic which started in August 1970 at Clacton on Sea showed that there was an unusual degree of respiratory distress followed by sudden death without the usual nervous symptoms, except in the later cases of the epizootic, when there seemed to be some modification of the disease. Although some cases were associated with enteric lesions these did not predominate, and in general the impression has been of a virus which affected the respiratory tract to an unusually high degree. This virus strain (designated Essex '70, was notable for the extremely rapid spread of the disease; since August 1970 there have been more than 7750 recorded outbreaks in Britain. At the same time disease caused by a virus later found to be of a similar nature occurred in Holland and

possibly in other countries in Western Europe. In the most recent comprehensive review of ND, Lancaster (1966) pointed out many ways in which the disease could be spread – for example, by the sale of infected carcasses, with subsequent infection of susceptible birds by the discarded unboiled giblets, or the movement of day-old chicks and point-of-lay pullets, subsequently known to have been in contact with the disease. In an area where the poultry industry is dense, both in the number of birds per site and in the total number of sites, the mechanism of spread may be obscured by the multiplicity of possible routes. These include the movement of supervisors and other itinerant employees, of birds, feed lorries, packing station crews and the free movement of sparrows and other wild birds. Lancaster also pointed out some experimental data suggesting evidence of airborne spread, by Idani & Seetheraman (1947) and by Delay, Deome & Bankowski (1948). Andrewes & Allison (1961) showed that in laboratory experiments cross-infection with NDV could take place via the air, but suggested that, because the effective distance in their experiments was short (less than 9 in), the virus was either carried in large particles which would not travel beyond this distance, or it was carried in small particles in which the virus was very labile. They also showed that there appeared to be a period of maximum infectivity, during the last 36 hr. of the infection. The air-spray technique of administering vaccine also shows that the virus will survive in the air, at least for short periods. Outbreaks during 1960–2 were shown by Smith (1964) probably to have been windborne as they followed the expected downwind deposition pattern of Pasquill's (1961) formulation.

The persistence of ND, and the ease with which it has spread in the recent outbreak, have been attributed to the high resistance of the virus to adverse conditions. This has raised once again the suggestion that some of the spread of the disease is by the airborne route. Therefore a series of experiments was carried out to obtain information on the following points:

(*a*) Do infected birds excrete virus into the air and, if so, when does the maximum output occur?

(*b*) What is the influence of relative humidity on the survival of the airborne virus?

(*c*) Can the virus remain infective in the open air?

(*d*) Can the virus be recovered from the air inside infected premises and also in the open air downwind of such premises?

(*e*) Does vaccination influence the excretion of virus from infected birds?

Information on all these points might be of considerable importance in the design of effective control measures.

MATERIALS AND METHODS

Organisms

The three strains of NDV used were Herts '33/56, Eastwood '67 and Essex '70. Suspensions of these strains were prepared at the Central Veterinary Laboratory, Weybridge (CVL), by harvesting the allantoic fluid from infected embryonated eggs two days after infection. These were stored at −70° C.

Bacillus subtilis var. *niger* spores (BG) were prepared as thick (approx 10^{11}/ml.) suspensions which were stored at 4° C. Just before use the small quantity required for a test was heated at 60° C. for 30 min. to eliminate any germinated spores or vegetative forms.

Escherichia coli (*EC*) MRE Strain No. 162 was grown in aerated culture vessels in tryptone broth containing glycerol (Elsworth *et al.* 1968).

Laboratory tests with aerosols

Preparation of suspension for spraying. The required quantity of heated BG suspension was centrifuged and the supernatant was removed from the pellet, which was then resuspended in the virus suspension. The final concentration of BG was approximately 10^{10}/ml.

Generation and storage of airborne particles. Aerosols were generated by spraying the virus suspensions containing BG with a Collison atomizer in a Henderson apparatus modified to operate over a range of relative humidities (Druett, 1969). Clouds were sampled immediately after generating (cloud age about 1 sec.) and after storage in the dark in a 500 l. rotating stainless-steel drum (Goldberg, Watkins, Boerke & Chatigny, 1958). Samples were collected from the storage drum after holding the cloud for 30, 60 and 240 min.

Cloud sampling. Samples were collected for 1 min. with raised impingers (May & Harper, 1957) at a flow rate of 11 l. per min. The collecting fluid was 10 ml. of nutrient broth containing 200 units of penicillin G.

Exposure to the open air

The microthread technique of May & Druett (1968), in which the airborne organisms are captured and held on ultrafine spider threads, was used. In many previous tests this method has been shown to give results similar to those found with airborne organisms. Microthreads were loaded with organisms by passing the cloud generated in the Henderson apparatus, as used for the laboratory tests, through sets of frames held in 'sows' (May & Druett, 1968). The exposure of the loaded microthreads was carried out in a ventilated unit designed by Druett (1971). This avoids the possibility of any virus particles that might be detached from the microthreads during exposure becoming airborne in the open air. In this unit the microthreads were exposed by pulling the open air through the exposure section at *ca.* 0·8 m./sec. The air was then passed through a high-efficiency filter before being discharged to the atmosphere. All the manipulations of the microthreads, once they were loaded with virus, were carried out in a safety cabinet vented via a high-performance filter.

Viability assessment

Bacteria. Suspensions, cloud and microthread samples were diluted in phosphate buffer (Anderson, 1966). The surface of each of four plates of tryptone agar was inoculated with 0·25 ml. volumes of the appropriate dilution. EC and BG colonies were counted after incubation of the plates overnight at 37° C.

Virus. NDV was assayed, usually immediately after collection, by inoculating

0·1 ml amounts of suitable dilutions in nutrient broth into the allantoic fluid of 9-day fertile eggs. The presence of virus in the inoculated eggs, after death of the embryo, was checked by haemagglutination tests. The virus titres were obtained by using the Spearman-Karber method (Finney, 1964).

Determination of viability of airborne organisms generated in the laboratory. The concentration of viable organisms in clouds or on microthreads is reduced by both physical loss and viable decay. To study loss of viability it is necessary to estimate the physical loss. The method used is to mix the bacteria or virus under test with a non-decaying tracer organism, BG. This will be subjected to the same physical loss as the test organism. The ratio of viable test organisms to viable BG in the starting suspension is equated to 100 % viability and the ratio of test organisms to BG in the cloud or microthread samples is expressed in terms of this ratio.

Air sampling

The Litton Large Volume (LV) air sampler (Litton Industries, Minneapolis, Minnesota, U.S.A.), which will concentrate particles from large volumes of air into a small volume of liquid, was selected for use, as very small numbers of virus particles were expected in the air. To obtain the maximum concentration of airborne material, it was necessary to recirculate the collecting fluid in the air sampler for periods up to 60 min. In this way the particles in 60,000 l. of air could be concentrated into about 30 ml. of fluid. Before using this technique it was necessary to know whether this long recirculation could cause any loss of viability to NDV collected early in the sampling period. To test this, a known quantity of Herts '33 strain virus was recirculated in the Litton air sampler. This was run in a virus-free atmosphere for 60 min. Samples were removed at intervals of 15 min. and these were titrated for NDV. There was no significant loss of virus during the 60 min. of running time.

Measurement of particle size

(*a*) *Cascade impactor* (May, 1945). Clouds were sampled at a flow rate of 17 l./min. onto slides coated with 5 % (w/v) gelatin in 10 % glycerol using a cascade impactor. The collected organisms were recovered by dissolving the gelatin–glycerol film in phosphate buffer at 37° C.

(*b*) *The multistage liquid impinger* (May, 1966). This sampler collects at 55 l./min. and is useful where the virus concentration is expected to be low. The particle-size distribution is as follows: top stage $> 6\ \mu$m., middle stage 3–6 μm. and bottom stage $< 3\ \mu$m. The collecting fluid was distilled water containing antibiotic.

RESULTS

Excretion of virus into the air by infected birds

These experiments were carried out using two strains of NDV. In the first experiment, five 6-week-old white Leghorn growers were inoculated intravenously with $10^{5\cdot3}$ ELD 50 of the Herts '33/56 strain and these donor birds were placed with 15 similar but uninoculated birds. These birds were held in a room $10 \times 10 \times 10$ ft.,

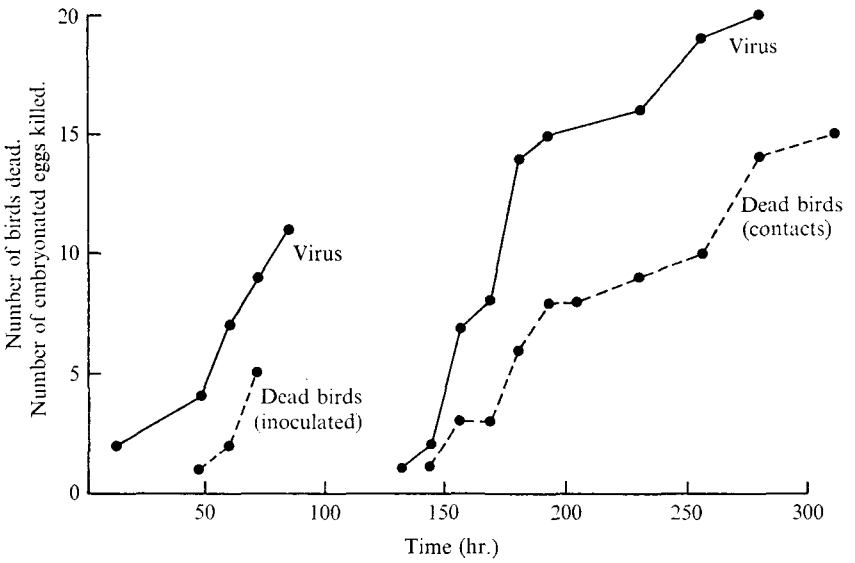

Fig. 1. Bird mortality and airborne virus recovered from contact experiment. (Virus concentration is expressed as the cumulative number of embryonated eggs killed when inoculated with 0·1 ml. (≡ 200 l. air) of fluid from the Litton Air Sampler.)

with positive pressure ventilation and a filtered exhaust system, maintained at 16° C. The relative humidity (R.H.) was between 30 % and 60 %. A chicken-wire partition, 6 ft. high, restricted the birds to two-thirds of the floor area. The Litton sampler was placed outside this partition at a height of 1 m. To reduce the amount of bacterial and spore contamination that would be generated from the normal litter, the floor area was covered with brown paper, which was replaced daily. This also reduced the chance of generation of airborne virus from the droppings. For the first 9 days of the experiment the air was sampled twice daily, starting at 09.00 and 20.00 hr. Thereafter the sampling was carried out only once per day. The Litton Sampler was primed with approximately 30 ml. of peptone–water broth containing penicillin G 5000 units/ml. and Fungizone* 20 μg./ml., which was recirculated through the sampler during the 60 min. sampling period. After each sampling period the sampler was removed from the room and cleansed with distilled water containing 1 % BRIJ 35 (non-ionic detergent, B.D.H. Ltd) and then washed with sterile distilled water. Dead birds were removed from the room at regular intervals.

In the second experiment, using the field strain Essex '70, 20 six-week-old Light Sussex birds were used. The air was sampled only twice, when the in-contact birds showed signs of disease, at 188 and 238 hr. after the start of the experiment.

Fig. 1 shows the results of the first experiment. The virus concentration is expressed as the number of embryonated eggs killed when inoculated with 0·1 ml. of the sample fluid from the Litton sampler, containing the particles from approximately 200 l. of air. As only small quantities of virus were recovered, it

* Amphotericin B, E. R. Squibb & Sons, N.Y., U.S.A.

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was not possible to estimate the virus content more precisely. Nevertheless it is obvious that virus is present in the air and that it is present at least 12 hr. before the death of the birds. The in-contact birds, which are presumably infected from the airborne virus released from the inoculated donor birds, started to die about 100 hr. after the release of virus from the donor birds. There is probably a similar effect in the last days of the experiment: the last five birds to die were probably infected with virus excreted by the first in-contact birds to show infection.

The second experiment showed that the Essex '70 strain of virus killed birds more rapidly. All 15 in-contact birds were dead after 238 hr. The virus concentration in the air samples was higher and estimations of virus titres by the method of Spearman & Karber (Finney, 1964) was possible. At 188 hr. 0·1 ml. of air-sampler liquid contained $10^{2\cdot45}$ ELD 50 and at 238 hr., when all the birds had died, it was $10^{1\cdot05}$ ELD 50. It was noted that the Light Sussex birds used in this experiment tended to huddle more than the white Leghorns used in the previous experiment with the Herts '33/56 strain.

The influence of relative humidity on the survival of airborne virus

Having established that the NDV could be recovered from the air of rooms containing experimentally infected birds, where the relative humidity (R.H.) was controlled between 30 % and 60 %, it was desirable to investigate the effect of a wider range of R.H. on the survival of airborne virus, as in commercial poultry houses the R.H. will vary widely from time to time. Three strains of NDV were used: Herts '33/56, Eastwood '67 and Essex '70. Aerosols of the three strains were generated and stored in the dark, at a variety of R.H. values, in a rotating drum and sampled as described in Methods.

As the suspensions of virus appeared to be much more viscous than the suspensions of bacteria usually used in this apparatus, it was first necessary to establish the size of the particles produced when allantoic fluid was sprayed from a Collison atomizer. Therefore clouds were generated from allantoic fluid containing BG in the Henderson apparatus and these were sampled approximately 1 sec. after generation, using a cascade impactor. These samples showed the following particle size distribution:

	Size limit of particles impacted (μm.)	% of total organisms recovered
Stage 1	6·0–2·0	0·3
Stage 2	2·2–6·0	5·2
Stage 3	1·0–3·0	35·7
Stage 4	0·5–1·5	58·8

Most of the particles generated were in the range of 0·5–3·0 μm similar to those usually found when aqueous suspensions of bacteria were used in this apparatus.

The results obtained using the three strains of NDV at various R.H. values are shown in Table 1. With all three strains, 30 % or more of the virus survived the initial spraying process. All three strains showed that 1 % or more of the virus remained viable after 4 hr. With the Essex '70 strain at 80 % R.H. viable virus was

Table 1. *Viability of three strains of NDV after holding in the rotating drum at 20° C. for various times and at different relative humidities* (R.H.)

Virus strain	R.H.	% viability after holding for 30 min.	60 min.	240 min.
Essex '70	80	36	12	6
	70	49	95	15
	60	40	33	9
	50	13	7	2
Herts '33/56	80	96	53	11
	80	67	—	11
	70	88	47	7
	60	18	14	3
	50	21	23	3
Eastwood '67	80	40	17	5
	70	64	38	10
	60	111	52	9
	60	34	39	4
	50	2	1	1

recovered after holding the cloud for 16 hr. but there were insufficient data to calculate accurately the amount present. Survival of all three strains at 50 % R.H. was lower than at 60–80 % R.H. There was insufficient evidence to show that any of the three strains survived better than the other two, under the conditions of this test.

The effect of exposure to open air on the infectivity of the virus

As an extension of the studies of survival of NDV in the rotating drum, experiments were carried out with virus particles held on 'microthreads'. It has been shown by Druett & May (1968), and by May, Druett & Packman (1969), that the toxicity of the open air varies from time to time when tested with a variety of micro-organisms exposed on microthreads. This germicidal property of the open air was attributed to the presence of an unstable Open Air Factor (OAF) which was rapidly lost when the air was enclosed. As an indicator for the toxicity of the open air, a reference micro-organism was included in our tests. The reference micro-organism, EC, has been used extensively for measuring the toxicity of the air and is considered to be an aerosol-robust micro-organism.

In the tests reported here the results obtained with three strains of NDV and the reference strain of EC are shown in Table 2. The survival of NDV was usually similar to that of EC exposed at the same time, indicating that NDV was not unduly sensitive to OAF. There was, as with the previous experiment, an initial loss of viable virus (approx 50 %), similar to that found with many other micro-organisms. For ease of presentation, the initial unexposed samples, collected at time 1 sec., have been given a nominal viability value of 100 %. Though it was not possible to select exactly similar conditions for comparing the different strains, a reasonable range of wind directions and R.H. values was used in tests with each of the strains. With all three strains, a significant quantity of virus survived for at least 30 min under all the conditions encountered.

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Table 2. *The viability of three strains of NDV held in the open air at various relative humidities* (R.H.) *and in winds from various directions*

(The control at 0 min. was given the nominal value of 100 %. The viability of *E. coli* 162 (EC) exposed at the same time is given for comparison.)

		Wind			Time of exposure		
Strain	R.H.	Direction	Speed (m./sec.)	Organism	30 min.	60 min.	Control, 60 min.
Essex '70	60	040°	25·5	NDV	62	31	36
				EC	26	8	79
	63	340°	17	NDV	24	8	4
				EC	10	3	84
	53	250°	20·4	NDV	20	0	18
				EC	9	3	41
	62	245°	17	NDV	22	5	28
				EC	6	3	74
	93	120°	8·5	NDV	11	0	46
				EC	5	0	82
Herts '33/56	75	290°	17	NDV	23	23	36
				EC	29	42	39
	95	190°	46	NDV	48	7	6
				EC	43	21	93
	70	300°	36	NDV	7	11	52
				EC	34	8	63
	78	070°	8·8	NDV	5	0	35
				EC	1	2	70
Eastwood '67	63	050°	18·8	NDV	30	—	38
				EC	3	—	76
	60	270°	18·8	NDV	212	59	189
				EC	20	2	103
	65	220°	24	NDV	1	< 1	4
				EC	28	13	81
	65	270°	27·2	NDV	51	3	9
				EC	36	6	130
	68	070°	13·6	NDV	5	0	35
				EC	6	1	68

Recovery of virus from the air inside and outside infected premises

Three farms at which there were reported outbreaks of Newcastle disease were used. Farm A (see plan, Fig. 2) consisted of eight poultry houses, each of which had been stocked with 8500 1-day-old chicks on 27–29 October 1970. On the day of sampling, 16 November 1970, 4000 birds were reported to be sick in the third house. On arrival at the farm a meteorological station was set up upwind of the houses. This recorded continuously the wind direction and speed, and air temperature, at a height of 2 m. above ground level, during the time that the air samples were taken, in this instance at night. Air sampling with a Litton LV sampler collecting at a rate of 1000 l./min. into 28 ml. of fluid was carried out, for

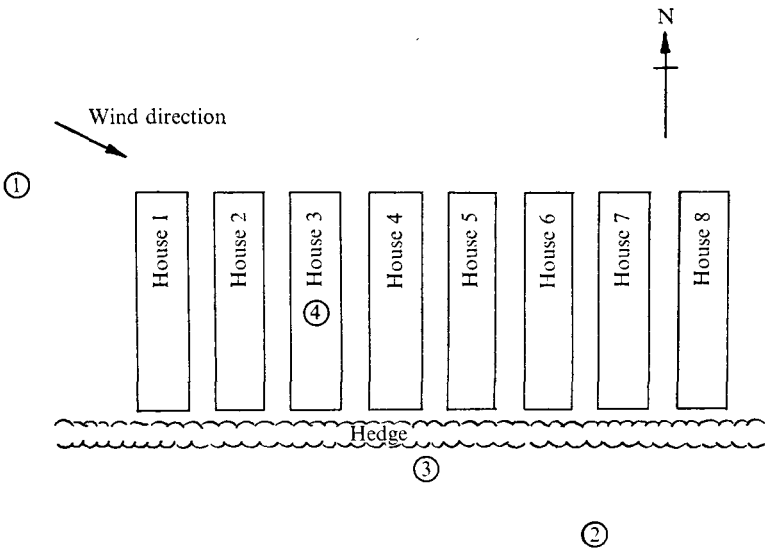

Fig. 2. Sampling sites (○) on farm A (not to scale).

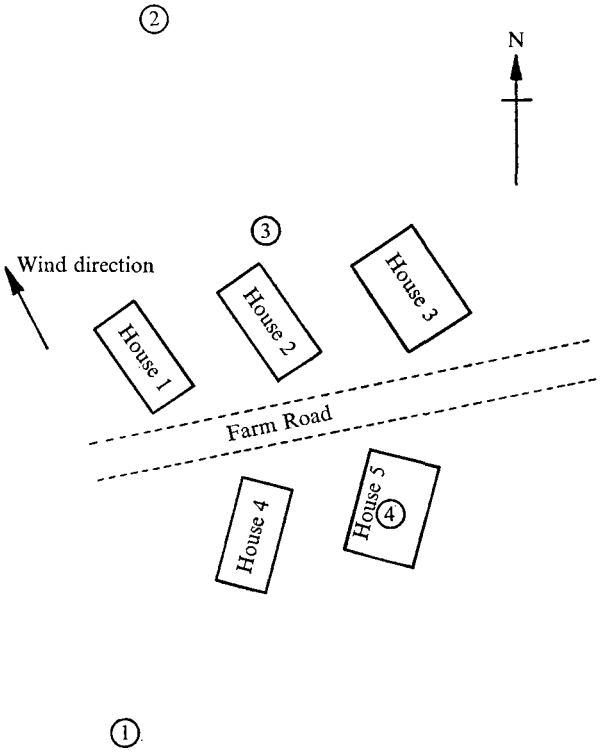

Fig. 3. Sampling sites (○) on farm B (not to scale).

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Table 3. *Amount of virus recovered from samples taken at various positions in and around two farms where there were outbreaks of Newcastle disease*

Sample position	Site no.*	R.H.	Temp. (°C.)	Virus recovered†
Farm A. (Samples taken at night, wind speed 0·4–0·7 m./sec., 280–295°; 8/8 cloud.)				
Upwind (21 m.)	1	94	6·1–6·9	Trace‡
Inside house	4	67	21·0–21·8	5·7
Downwind (23 m.)	3	96	4·2–4·4	2·95
Downwind (55 m.)	2	92	5·3–5·5	2·0
Farm B. (Samples taken in daylight, wind speed 2·9–3·9 m./sec., 130–145°; 6/8–8/8 cloud)				
Upwind (101 m.)	1	82	8·3	Trace‡
Inside house	4	70	15–15·5	5·28
Downwind (64 m.)	3	79	8·3–8·6	1·45
Downwind (165 m.)	2	84	7·8–8·3	Nil

* See Fig. 1.
† Virus titre, $\log_{10}$ ELD 50 per 33,000 l. of air.
‡ Trace = too low for accurate estimation.

30 min. each at (1) upwind of the poultry houses beside the meteorological station, (2) 55 m. downwind of the farm buildings (as far as it was possible to go without encountering obstructions between the farm building and sampling station), (3) 23 m. downwind of the building containing the infected birds, and (4) inside the house containing the infected birds. The height of the sampler above ground level was 1·7 m. when used in the open air and 0·5 m. when used inside the building. After sampling, the collecting fluid was drained off and stored at 4° C. before assessment. The machine was then flushed out with 0·1% BRIJ 35 solution followed by sterile distilled water and then refilled with collecting fluid.

At the second farm, B (Fig. 3), houses (1) and (2) had been stocked on 5 October 1970 with 7500 1-day-old chicks, house (3) with 13,000, house (4) with 9000 and house (5) with 14,000. Excess deaths were first noticed in houses (3) and (5) on 13 November; by 17 November, when the air samples were taken during daylight, 11,000 birds had died in house (5) and the moribund and morbid birds were showing signs of respiratory distress. Air sampling was carried out as on farm A. The sampling sites and their distances are shown in Fig. 3 and Table 3. At this farm the sample taken inside the building (sample (4)) was taken in two parts: (1) for 10 min. followed by (*b*) a 20 min. sample, as it was thought possible that the very high level of ammonia in the air might have a deleterious effect on the virus being circulated in the sampling fluid. At both farms the inside samples were taken with the ventilating fans off and the outside samples with the fans on.

The results of the virus recoveries in samples taken at farm A are shown in Table 3. Sample (4), taken inside the infected house, contained $10^{5·7}$ ELD 50 virus units in 33,000 l. of air, samples (2) and (3), collected downwind of the poultry houses, also contained significant amounts of virus. The sample taken upwind of the premises (sample (1)) contained a very small amount of virus insufficient for accurate estimation. It is possible that, as the sampler had been previously used

Table 4. (*a*) *The size distribution of the particles carrying Newcastle disease virus recovered from air inside a poultry house at farm C*

Particle size (μm.)	Stage of multistage sampler	Virus recovered*	
		At litter level	1·7 m. above floor
> 6	Top	3·39	3·11
3–6	Middle	3·14	3·31
< 3	Bottom	1·68	1·97

* Virus titre = $\log_{10}$ ELD 50 per 1000 l. of air.

Table 4. (*b*) *The size distribution of particles carrying bacteria recovered from air inside normal poultry houses*

Particle size (μm.)	Stage of multistage sampler	% of total bacteria recovered (at litter level)	
		House 1	House 2
> 6	Top	94	83
3–6	Middle	5	11
< 3	Bottom	1	6

earlier in the day at CVL, this small amount of NDV could be a carry over caused by insufficient washing of the sampling apparatus.

Similar amounts of virus were recovered from air samples at farm B (Table 3). Once again the upwind sample contained a small amount of virus, thought to be due to contamination. The downwind sample, collected 165 m. away from the infected premises, did not yield any NDV. As this sample was collected during an overcast day, the lack of viable virus in the sample might have been due to the virucidal effect of daylight.

At farm C the houses had been stocked on 3 January 1972 with 12,750 1-day-old chicks. These had been vaccinated on arrival with infectious bronchitis vaccine. On days 3 and 14 they received the vaccine strain Hitchner B1 virus in their drinking water, and on days 28 and 31 La Sota virus was also given in their drinking water. Air samples were taken on day 37 when the birds were dying in large numbers. By the end of the following week when the outbreak was over, some 40 % of the birds had died. At this farm the air was sampled using a multistage liquid impinger (May, 1966), collecting at 55 l./min. in an attempt to estimate the particle size of the airborne virus. Air samples were collected for 30 min. inside the building with the fans off: sterile distilled water containing penicillin–streptomycin (200 units/ml.) was used as the collecting fluid. Immediately after sampling the fluid from each stage was recovered and stored at 4° C.; it was titrated immediately on returning to the laboratory 4·5 hr. later. Virus recoveries are shown in Table 4(*a*); the quantities of virus recovered when sampling at litter level and at 1·7 m. above the floor level are similar. It can be seen that nearly all of the virus is collected in the top two stages, equally divided between particles from 3–6 μm. and those above 6 μm. Comparable concentrations of virus were recovered from

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Table 5. *Comparison of the amounts of NDV recovered from vaccinated and non-vaccinated birds*

No. of days after challenge		Birds		Virus recovered*
		Live	Dead	
4	Vaccinated	90	0	2·72
	Non-vaccinated	86	4	4·66
5	Vaccinated	90	0	2·24
	Non-vaccinated	47	43	4·18
6	Vaccinated	90	0	2·20
	Non-vaccinated	23	67	2·87

* Virus titre, $\log_{10}$ ELD 50 per 1000 l. air.

the air at this farm using the multistage sampler as at the other two farms when the Litton Sampler was used. Some air sampling experiments (unpublished work), carried out in normal non-infected poultry houses, showed that the bacterial flora of the air in a house was carried mostly on the larger particles (Table 4*b*).

The influence of vaccination of infected birds on excretion of virus into the air

Two batches of 90 Ross birds were used. They were kept in separate environmentally controlled rooms with a volume of approximately 1000 ft.^{3} each. Batch A birds were inoculated with La Sota virus in their drinking water when 21 and 35 days old. Batch B birds were not vaccinated. When both batches of birds were 56 days old they were each challenged with $4 \times 10^{10 \cdot 6}$ ELD 50 Essex '70 virus, diluted to 25 ml. with distilled water and sprayed from a Humbrol aerosol propellant generator. From the 4th to the 6th day after challenge, the air in both holding rooms was sampled each day for 30 min using a multistage liquid impinger. The ventilating fans were turned off during the sampling period. The collecting fluid was distilled water containing streptomycin, and the samplers were at floor level. The fluid was recovered directly after sampling and titrated immediately. The results are shown in Table 5 and it is obvious that a much larger amount of virus is excreted by the non-vaccinated birds. The amounts of virus recovered per bird per hour, based on the number of birds alive during sampling, were about the same for each day with the vaccinated birds. For the unvaccinated birds it fell for the last day, but there was still more virus excreted at this stage than from the vaccinated birds. There were a number of non-specific deaths in the eggs used for NDV titration during the first part of the experiment: this was overcome in later samples by increasing the concentration of streptomycin in the collecting fluid. Although these non-specific egg deaths affected the precision of the titration, the benefits of vaccination were so obvious that the conclusions were not affected.

DISCUSSION

The results of laboratory experiments and field sampling reported here make a strong case for implicating the airborne travel of viable Newcastle disease virus

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(NDV) as a means of spreading the disease during large outbreaks of Newcastle disease such as we have recently encountered in Great Britain.

A preliminary laboratory experiment with the Herts '33/56 strain, in which an attempt was made to reduce the amount of possible regeneration of virus from bird droppings by replacing the usual straw or sawdust litter with paper sheets, showed that virus could be recovered from the air in poultry houses containing experimentally infected birds. Although only small quantities of virus were recovered, it was obvious that it was excreted into the air at least 12 hr. before the first deaths from the disease. The in-contact birds started to die about 100 hr. after the virus was first found in the air, and the amount of virus recovered was in proportion to the number of infected birds. A second experiment using the Essex '70 strain of virus (isolated in the recent outbreak in U.K.) gave similar results, but in this case the progress of the disease was more rapid and the amount of virus recovered from the air was greater. At 188 hr., when the first infected birds died, the virus concentration was $10^{2.45}$ ELD 50 in approximately 200 l. of air.

Once it had been established that viable virus was excreted into the air, it was necessary to carry out some investigations of the survival of the virus within the range of temperature and R.H. commonly encountered in Britain. These experiments were carried out in a closed container. Because of previous findings that there were present in the open air certain toxic factors capable of reducing the viability of airborne micro-organisms, they were followed by a series of tests where the virus was exposed on microthreads to the open air. This method has been shown to give results similar to those obtained with cells carried on free airborne particles. Although the range of meteorological conditions could not be selected in advance, exposures were carried out in a variety of conditions that might be expected after dark in Britain. From the results of these experiments it was obvious that the virus could survive for a sufficient length of time to make the airborne spread of the disease possible.

Having established that the virus could survive in the open air it was desirable to attempt to recover viable virus from the air in the vicinity of infected premises. On these occasions, sampling apparatus was taken to farms where outbreaks of Newcastle disease had been reported. Samples of air were collected inside the infected houses and at two of the farms samples were also collected outside at distances up to 165 m. downwind of the premises. Appreciable quantities of virus were recovered from the air inside the houses at all three farms and less but still measurable quantities at distances up to 64 m. downwind. The sample taken at 165 m. downwind at farm B yielded no virus, but it should be remembered that this sample was collected during daylight.

Finally, an attempt was made to investigate the effect of vaccination on the output of airborne virus from infected birds. From the results it is clear that a much larger amount of viable virus is excreted into the air by the unvaccinated birds. The protection conferred by the vaccine is obvious, but nevertheless it is significant that the protected infected birds did excrete quite appreciable quantities of virus into the air.

Though fairly conclusive, these investigations pose many extra questions. For

instance how does the size of the particle in which the virus is airborne affect its survival? It has been shown (Zalko-Titanenko, 1965; May, 1966) with other micro-organisms that viable decay of airborne cells occurs more rapidly in small particles than in large ones. Where do the particles of different size lodge in the respiratory tract of birds? With other animals it has been shown that the size of the particle carrying airborne micro-organisms is of importance in determining where they lodge in the respiratory tract and the number of organisms needed to initiate infection by the respiratory route (Druett, Henderson, Packman & Peacock, 1953). The respiratory tract of birds is quite different from that of other animals and there seems to be no information on penetration of particles into it, or the number of NDV particles needed to infect by this route. Our investigations indicated that most of the airborne virus was carried in particles exceeding 3 μm. in diameter. Is this the size of the particles excreted or is it a secondary aerosol after absorption onto particles of feather or litter? Is the particle size of the excreted virus affected by vaccination? What is the optimum particle size for the economical and efficient distribution of airborne vaccine?

Studies along most of these lines are now being planned or are already in progress.

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