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OCCURRENCE OF AVIAN INFECTIOUS BRONCHITIS VIRUS IN THE TISSUES OF EXPERIMENTALLY INFECTED CHICKENS

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SUMMARY

Eighteen chickens 4 weeks of age that had been experimentally infected with infectious bronchitis virus (IBV) were killed, two each day, from 1 to 9 days after inoculation. IBV was isolated in chick embryos from the trachea (14 times), lung (11), heart and kidneys (10 each), spleen and liver (7 each) and blood (once). The virus was recoverable on the ninth day after inoculation.

The virus was isolated with a similar frequency from 8 birds that died from 2 to 10 days after inoculation. The tissue samples were held at —15°C, for as long as 11 days, before inoculation into chick embryos. IBV was readily recovered from this material.

I. INTRODUCTION

Infectious bronchitis has been considered to be primarily a respiratory disease, with attendant growth retardation in young birds and depression of egg production in adults (Roekel *et al.* 1950). Viruses similar to infectious bronchitis virus (IBV) have been shown recently to be the cause of avian nephrosis in America (Winterfield and Hitchner 1962) and Australia (Cumming 1963). Kawakubo, Miyamoto, and Nakamaru (1961) were able to isolate a Japanese strain of IBV from the lungs, tracheal mucus, intestinal contents, blood, brain, spleen, liver and kidney of experimentally infected birds.

This paper reports the frequency of occurrence of an Australian strain of IBV in the respiratory tract, blood and visceral organs of chickens for a 10-day period following inoculation.

II. MATERIALS AND METHODS

Thirty Australorp chickens 4 weeks of age were used. After obtaining blood samples from all chickens, 28 of them were inoculated intratracheally with 0·2 ml of the N4454 strain of IBV (Newton and Simmons 1963; Gartner, Newton, and Burton 1966). The virus was in its seventeenth chick embryo passage and the titre of the inoculum was $10^{6\cdot 0}ELD_{50}$/ml.

The birds were housed individually in 30 small stainless-steel cages in an isolation room. Each cage was enclosed in an air-tight plastic bag. Air from outside the room was pumped, by two diaphragm pumps (Autometric pumps, type VMY), into a central distribution unit with 30 separate outlets to the individual cages. Exhaust air was similarly extracted with a water-jet pump which discharged into an underground drain. The two uninoculated birds were sealed into their units immediately before the virus was used.

The cages were numbered and these numbers were randomized. Each morning fresh feed, water and bedding were added for one bird. After this was done another bird was removed for post-mortem, in the randomized cage sequence, and the empty unit was resealed. This process was repeated each afternoon.

Birds were autopsied in a laboratory remote from the experimental area. The neck and abdomen were swabbed with 70% alcohol. The carotids and spinal cord were cut with sterile scissors, care being taken to avoid the trachea. The bird was bled out as fully as possible and 0·5 ml of blood, without added anticoagulant, was injected allantoically into each of four 8-day chick embryos before clotting occurred.

The bird was taped out, with dorsal aspect down, on a stainless-steel tray. Using separate sets of sterile scissors and forceps for each of the following procedures, the skin was reflected, the trachea was dissected out and divided lengthwise, the abdomen and thorax were opened, and the lung, liver, spleen, heart and kidneys were sampled. A portion of the trachea and approximately 0·5 g of each organ were placed in separate sterile mortars and macerated as a 10%

suspension in frozen antibiotic diluent. This was allowed to stand for at least 15 min, the fluid centrifuged at 500 *g* for 5 min and 0·5 ml of the supernate injected allantoically into each of four 8-day chick embryos. Allantoic fluid from chick embryos inoculated with blood and organ suspensions was passaged after 2 days' incubation, the second passage re-passaged after 2 days' incubation, and the third passage survivors examined for dwarfing after 8 days' incubation. All dead embryos were cultured on blood agar.

The antibiotic diluent consisted of Hanks' balanced salt solution (Hanks and Wallace 1949) incorporating 0·5% lactalbumin hydrolysate, 0·01% Difco yeast extract and 2 x 10^3 i.u. of C.S.L. sodium penicillin G, 2 mg of Glaxo streptomycin sulphate, 2 mg of Sigma Kanamycin sulphate and 0·5 mg of Squibb amphotericin B per ml. This was adjusted to pH 7·0 with 2·8% sodium bicarbonate solution and stored at —20°C.

A longitudinal section of the trachea, the posterior part of the left lung, the middle lobe of the liver, one half of the spleen, the apex of the heart and the poles of each kidney were fixed in Zenker's fluid. After fixation, these tissues were embedded in paraffin and sections were cut at 6 microns and stained with haemotoxylin and eosin.

Birds found dead in the cages were sampled as above, except that material for attempted isolation was held unmacerated in ¼-oz bottles at —15°F until the end of the experiment. As the sections of tissue from these birds showed considerable evidence of post-mortem degeneration, they were excluded from the study.

Pre-inoculation sera were screened for neutralizing antibody to IBV by incubating undiluted serum with 100 ELD_{50} of the N4454 virus for 2 hr at room temperature. Then 0·5 ml of the virus-serum mixture was inoculated allantoically into each of six 8-day chick embryos.

III. RESULTS

No neutralizing antibody was found in pre-inoculation serum samples from any of the birds. The frequency of isolation of IBV from the organs of the 18 chickens killed from 1 to 9 days after inoculation is shown in Table 1. It was possible to isolate the virus from some sites 9 days after inoculation. The virus was most frequently isolated from the trachea. Only one isolation was made from the 13 blood samples examined. Similarly, in the eight infected chickens found dead in their cages from 2 to 10 days after inoculation (Table 2), the highest number of isolations was from the trachea.

TABLE 1

ATTEMPTED ISOLATION OF IBV FROM EXPERIMENTALLY INFECTED CHICKENS KILLED FROM 1 TO 9 DAYS AFTER INOCULATION

Bird No.	Post-inoculation Period (days)	Virus Isolation						
		Blood	Trachea	Lung	Liver	Spleen	Heart	Kidney
1	1	—	—	—	—	—	—	—
2		NS	+	+	—	+	+	+
3	2	—	+	+	—	—	—	—
4		—	+	—	—	—	—	—
5	3	—	+	+	+	+	+	+
6		—	+	+	+	+	+	—
7	4	—	—	—	—	—	—	—
8		—	—	—	—	—	—	—
9	5	C	+	+	+	—	+	+
10		NS	+	+	+	+	+	+
11	6	—	+	+	+	+	+	+
12		—	+	+	+	+	+	+
13	7	+	+	+	+	+	+	+
14		—	+	—	—	—	—	+
15	8	NS	+	—	—	—	—	—
16		—	+	+	—	—	+	+
17	9	C	+	—	—	—	—	+
18		—	—	+	—	—	+	—
Total isolations ..		1	14	11	7	7	10	10

C = Contaminated

NS = No sample

One control bird died on day 4 and the other was killed on day 14. The virus was not isolated from either control, or from one chicken killed on day 1 or two chickens killed on day 4. No abnormalities were seen in histological sections of the respiratory tract or visceral organs of these five birds.

Some degree of hyperaemia and oedema of the mucosa with concurrent proliferation of lymphoid elements and decrease in prominence of goblet cells was observed in sections of the trachea from all inoculated birds that were killed for autopsy except numbers 1, 7, 8 and 14. These changes were most apparent in numbers 9, 10, 11 and 12, and these four chickens were accompanied by almost complete loss of cilia on the surface epithelium.

TABLE 2

ATTEMPTED ISOLATION OF IBV FROM EXPERIMENTALLY INFECTED CHICKENS FOUND DEAD FROM 2 TO 10 DAYS AFTER INOCULATION

Bird No.	Post-inoculation Period (days)	Length of Storage of Tissues* (days)	Virus Isolation					
			Trachea	Lung	Liver	Spleen	Heart	Kidney
19	2	11	+	+	−	−	−	+
20	2	11	+	+	+	−	−	−
21	2	10	+	−	−	−	−	−
22	3	9	+	+	+	+	+	+
23	3	8	+	+	−	+	+	+
24	3	8	+	+	+	+	+	+
25	9	1	+	+	+	+	+	−
26	10	0	C	C	−	+	−	+
Total Isolations			7	6	4	5	4	5

C = Contaminated

* = Tissues were stored at −15°C until isolation was attempted

None of the kidneys showed macroscopic abnormalities and microscopic examination revealed an increase in mononuclear cell infiltration in the kidneys of one bird, number 16. There was hyperplasia of the peribronchial lymphoid nodules in lung sections from birds 10 and 16. No lesions were seen in sections of the spleen, liver and heart of infected chickens.

IV. DISCUSSION

The finding that IBV could be isolated from experimentally infected chickens for as long as 10 days after inoculation is in accord with the results of Kawakubo, Miyamoto, and Nakamaru (1961), who were able to recover the virus for as long as 21 days after inoculation. The fact that IBV was only isolated from one of 13 blood samples indicates that prolonged viraemia was not a feature of this experimentally induced disease. Therefore it seems reasonable to assume that IBV may localize, at least temporarily, in the liver, spleen, heart and kidneys. The isolation of virus from all samples from bird 13 may have been a result of presence of viraemic blood in the tissues.

Chick embryo passage is known to decrease the virulence of IBV for chickens (Cunningham 1957). This strain of IBV has been passaged 17 times in chick embryos and now does not normally kill young chickens (Gartner, Newton, and Burton 1966), although it was originally virulent (Newton and Simmons 1963). The death of birds confined in these isolation units may have been associated with the extremely high humidity inside the plastic bags.

Microscopic lesions in the trachea were similar to those described by Hofstad (1945). A greater degree of abnormality may have been observed in lung sections if they had been taken from the antero-ventral area, where, according to Hofstad (1945), most lesions are found.

No explanation is offered for the failure to isolate virus from the two birds killed on day 4.

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