Experimental Infection with Avian Infectious Bronchitis Virus (Kagoshima-34 Strain) in Chicks at Different Ages

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ABSTRACT. Chicks at 2, 4 or 6 weeks of age were experimentally infected individually with a nephrosis/nephritis-causing avian infectious bronchitis virus (IBV) strain Kagoshima-34. The susceptibility of chicks in each group to the infection was compared, based on the clinical signs, excretion of virus in the faeces and antibody titres in the serum. The results showed that although all chicks appeared to be susceptible to IBV infection, the most severe clinical response was observed following infection at 2-week-old. Likewise, whilst the virus was also recovered from the faeces of all the chicks infected, the duration of viral excretion was longest in the 2-week-old chicks. A high antibody titre was detected at 4 weeks post infection (PI) and was maintained for at least another 16 weeks in the 4- and 6-week-old chicks. In contrast, a low antibody titre was detected only between 8 to 12 weeks PI in the 2-week-old chicks. Thereafter, no antibody was detected despite the presence of clinical signs.—KEY WORDS: antibody production, avian infectious bronchitis virus, clinical sign, long term infection, nephrosis/nephritis.

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For the control of avian infectious bronchitis (IB), various kinds of live and killed IB vaccines have been widely used in most chicken farming areas in the world. However, it is well known that such vaccines are not necessarily always effective. In particular, chicks which have a considerable titre of antibody against avian infectious bronchitis virus (IBV) are not necessarily protected from infection with virulent IBV [7, 11, 23, 24]. Thus, for the control of this disease, it seems necessary to make a more detailed analysis of the properties of IBV.

IBV changes its antigenicity following *in vivo* [16, 20] and *in vitro* [19] passages. Otsuki *et al.* [20] reported that IBV changes its organ tropism as well.

It has been generally thought that susceptibility of chick to infection with IBV varies with age. Most workers believe that younger chicks are more susceptible to IBV infection than older ones [2, 10, 15, 26] although a contradicting result has also been reported [17].

It has been observed in the field that IBV causes persistent infection in young chicks [9, 12, 22]. For example, Alexander and Gough [1] and Alexander *et al.* [2] reported that chicks experimentally infected with IBV excreted virus in the faeces for more than 20 weeks with corresponding serum antibody production.

The present investigation was carried out to compare the clinical signs, virus excretion in the faeces and serum neutralising antibody response of chicks of various ages experimentally infected with a field IBV strain, Kagoshima-34 (K-34). Since it is known that IBV causes persistent infection in chickens [2, 8, 10, 22], observation of and sampling from chicks infected with IBV was done for 20 weeks after inoculation with IBV.

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MATERIALS AND METHODS

Viruses: A field IBV isolate, strain K-34 was used in this investigation [21]. This virus was isolated from the kidneys of a chicken that died of nephrosis/nephritis in 1981. Two other strains of IBV, Beaudette-42 (Be-42) and Connecticut A-5968 (A-5968) were used as antigens. All viruses were titrated in tracheal organ cultures (OC).

Organ culture: Tracheal rings of chicken embryo were cultured by the method described by Cook *et al.* [5].

Experiment 1: For each age group of 2, 4 or 6 weeks, four SPF White leghorn chicks were inoculated intratracheally with IBV strain K-34 ($10^{6.0}$ EID_{50}). These 3 groups of chicks tested were housed on wire and were kept in separate isolated rooms throughout the investigation period of 20 weeks. All chicks were observed for clinical signs everyday. The faeces of each group were collected every week for IBV recovery and blood were taken every other week for titration of neutralising antibody.

Experiment 2: Thirty four 2-week-old SPF chicks were inoculated with IBV K-34 by the same manner as in experiment 1. Every other week, 3 chicks were bled, killed and examined post mortem to investigate macroscopic lesions in the trachea, lungs and kidneys.

Virus recovery: Virus recovery from the faeces excreted by infected chicks was attempted using the previous method [18] in SPF embryonated eggs. Pooled faecal samples of each group were prepared as a 20% w/v suspension in PBS containing 10,000 units/m*l* penicillin and 10 mg/m*l* streptomycin. All the samples were passaged 6 times before being considered as negative.

Serum neutralisation test: Sera from infected chicks in experiments 1 and 2 were examined individually for IBV-neutralising antibodies to strains K-34, Be-42 and A-5968 using log $10^{2.0}$ median ciliostatic doses (CD_{50}) of

virus in a neutralisation test in OC as described previously [4]. Titres were calculated by the method of Reed and Muench [25].

RESULTS

Experiment 1

(1) Clinical signs

The results obtained are summarised in Table 1. All two-week-old birds showed severe respiratory signs between 3 and 6 days PI and mild ones again at 8, 13 and 14 weeks PI. Diarrhoea began 3 days PI, lasted for another 14 weeks and was observed again at 19 weeks PI. Two chicks died at 9 and 12 days PI, both showing lesions typical of IB infection in the kidneys. Although the 4- and 6-week-old infected chicks showed respiratory signs until 2 weeks PI and 6 days PI, respectively, and occasionally again thereafter, these were milder than those in the 2-week-old chicks. The diarrhoea observed in the 4- and 6-week-old chicks was intermittent and of short duration, unlike that seen in the 2-week-old chicks. No chick died in the 4- and 6-week-old within the observation period of 20 weeks.

(2) Virus recovery from faeces

As shown in Table 2, chicks in all age groups consistently excreted IBV in their faeces for at least 6 weeks. The 2-, 4- and 6-week-old chicks excreted IBV intermittently until 19, 18 or 16 weeks PI respectively. In almost all cases, at least 3 blind passages in embryos were needed to confirm virus recovery.

(3) Neutralising antibody production

In this test, the homologous strain K-34 and two other strains of IBV, Be-42 and A-5968, were used as antigens. As shown in Fig. 1, serum neutralising antibody was detected at 4 weeks PI in both the 4- and 6-week-old infected chicks and persisted throughout this investigation. In both age groups, the antibody titres reached their peak at 10 weeks PI. The peak titre for the 4-week-old chicks was 2.9 log 10 and for the 6-week-old chicks, 3.2 log 10. Antibody against Be-42 and A-5968 strains was also detected, however, the titres were lower than those against the homologous K-34 strain.

In the 2-week-old chicks, neutralising antibody levels were low (the highest titre was 2.0 log 10 at 8 weeks PI) and antibody was detected only from 8 to 12 weeks PI, although diarrhoea continued to be observed in these chicks. No neutralising antibody was detected against either the Be-42 or A-5968 strains.

Table 1. Clinical signs in SPF chicks infected with IBV strain K-34. (Experiment 1)

	Respiratory signs			Diarrhoea		
Age (weeks)	2	4	6	2	4	6
Days (PI)						
1	−	−	−	−	+	+
2	−	−	−	−	+	−
3	++	+	+	++	+	−
4	++	+	+	++	+	−
5	++	+	+	++	−	−
6	++	+	+	++	−	−
Weeks (PI)						
2	−	+	−	+	+	−
3	−	−	−	+	−	+
4	−	−	−	−	−	−
5	−	−	−	−	+	−
6	−	−	+	+	−	−
7	−	−	−	+	−	−
8	+	−	−	+	−	−
9	−	−	+	+	−	−
10	−	−	+	+	−	−
11	−	+	−	+	−	−
12	−	+	−	+	+	−
13	+	−	−	+	+	+
14	+	+	−	−	−	−
15	−	−	−	+	+	−
16	−	−	−	−	−	−
17	−	−	−	−	−	−
18	−	−	−	−	−	−
19	−	−	−	+	−	−
20	−	−	−	−	−	−

\+ : With clinical signs
++: With severe clinical signs
− : Without clinical signs

Table 2. Virus recovery from the faeces excreted by chicks following intratracheal inoculation with the K-34 strain of IBV

Week(s) post inoculation	Age when inoculated (weeks)		
	2	4	6
1	+4	+3	+5
2	+4	+3	+4
3	+3	+4	+4
4	+5	+5	+6
5	—	—	—
6	+4	+3	—
7	—	—	—
8	—	—	—
9	—	—	+3
10	—	—	+2
11	—	+3	+5
12	—	—	—
13	+5	—	—
14	—	—	—
15	+3	+3	+5
16	+3	—	+5
17	—	+3	—
18	+4	+6	—
19	+6	—	—
20	—	—	—

+: Virus recovered after the indicated number of passages.
−: No virus recovered.
The parent virus used to infect these chicks causes an apparent IBV lesions of curling and stunting for chick embryo.

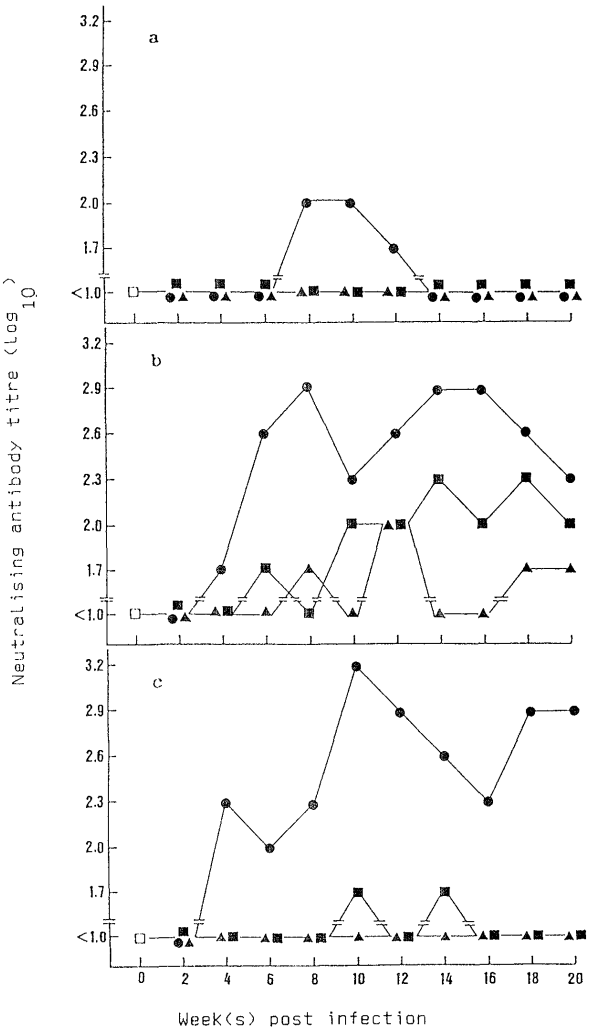

Fig. 1. Serum neutralising antibody titres of 2-(a), 4-(b), and 6-week-old (c) infected chicks against 3 different IBV strains, following intratracheal inoculation with IBV strain K-34 (Experiment 1). K-34=●, Be-42=▲, A-5968=■.

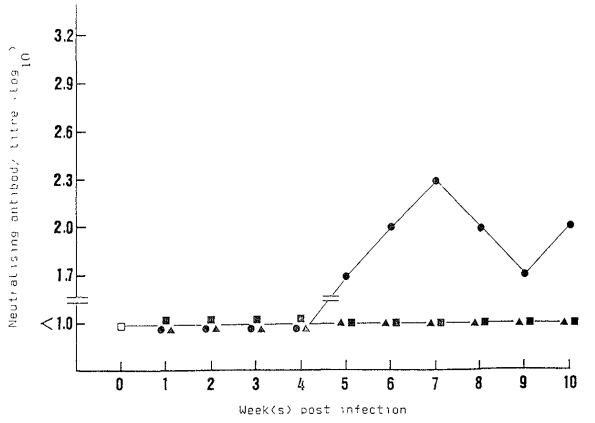

Fig. 2. Serum neutralising antibody titres of 2-week-old infected chicks against 3 different IBV strains, following intratracheal inoculation with IBV strain K-34 (Experiment 2). K-34=●, Be-42=▲, A-5968=■.

Experiment 2

The results obtained in experiment 1 showed that although the clinical signs in the 2-week-old chicks were more severe than those in the 4- and 6-week-old chicks, their antibody production was lower. Experiment 2 was done to confirm the results obtained in the experiment 1. Thirty five 2-week-old chicks were infected with the same virus as that used previously. These chicks were observed carefully for clinical signs until 10 weeks PI. Every two weeks, 3 chicks were bled and sacrificed, and their trachea, lungs and kidneys examined for macropathological lesions.

The results showed that all infected chicks manifested typical respiratory signs of IBV infection for 7 days and diarrhoea was consistently observed throughout the experimental period (data not shown). By 4 weeks PI, all kidneys of the infected chicks were pale and swollen. Exudate in the trachea was observed in all chicks examined until 10 weeks PI. No severe lesions were observed in the lungs. Neutralising antibody against only the K-34 strain was detected from 5 weeks PI, but its titre was low (Fig. 2). No mortality was observed in this experiment.

DISCUSSION

In this investigation, all chicks infected with IBV K-34 strain showed respiratory signs, which confirms previous reports that IBV strains causing nephrosis/nephritis can be isolated not only from the kidneys but also from the respiratory tissues [1, 13, 19, 20]. It is evident that IBV K-34 strain also caused persistent infection in young chicks at or below 6 weeks of age as in the case with H-120 and Australian T strains [1, 2]. Considerable mortality was observed in the 2-week-old chicks infected with the K-34 strain; this was not observed in the 2 older groups. Furthermore, the clinical signs, particularly diarrhoea, observed in the 2-week-old chicks was more severe and of longer duration than that in the 4- and 6-week-old ones. This finding suggests that younger chicks are more susceptible to IB-induced nephritis/nephrosis than older ones, which is contrary to the report of MacDonald *et al.* [17] who found that 3-week-old chicks were more resistant to infection with IBV causing nephrosis/nephritis than day-old, or 10 to 12-week-old chicks.

In the 2-week-old chicks, serum neutralising antibody was not detected until 6 weeks PI (Experiment 1), although 2 chicks from this group died at 9 and 13 days PI, and the surviving chicks continued to show severe clinical signs. Antibody was not detected beyond after 14 weeks PI, although these chicks still showed some clinical signs. Antibody production in the 2-week-old chicks seems to be less than that in the 4- or 6-week-old ones. It is difficult to analyse this phenomenon at present and thus, it needs further investigation. However, it is possible that the immunocytes of the 2-week-old chicks were still immature, hence, did not respond sufficiently to the infection. The lymphoid organs such as the thymus and Harderian

glands may also be immature at this age and their cells, particularly those of the thymus, may have been partly damaged as a consequence of the IB infection, thus affecting both T and B cell activity.

In a previous report, it was demonstrated that IBV K-34 changes its organ tropism and antigenicity [20]. In this investigation, K-34 strain also changed its antigenicity slightly and its pathogenicity for chick embryo was easily lost (Table 2) as it took several egg passages before virus recovery from the faeces excreted by IBV infected chicks was confirmed. It is suggested that the K-34 IBV strain consists of various different subpopulations [19] and that a subpopulation that is pathogenic for chick embryos became less pathogenic or possibly died out and other subpopulations grew better during replication in the epithelial cells of the chicken intestine.

It has been reported that a higher rate of infection with IB when chickens were housed on litter than on wire [3]. In the present investigation, although infected birds were housed on wire and were kept in separate rooms throughout the duration of experiment, the possibility of reinfection could not be entirely eliminated.

It is well known that chickens infected with IBV at a very young age tend to become non egg-producing hens [6]. In the field, antibody production by very young chicks infected with IBV may not be very high. Thus, IBV easily causes persistent infection to young chicks. Such IBV-infected chicks are a continuing source of IBV infection and some of them also might become non egg-producing hens.

Since IBV has many target tissues in the chickens [6, 8, 12, 14], several fluids other than serum should to be titrated for their IBV antibody content in order to get further information about the reaction of chicks to IBV infection. Titres of IBV antibody detected in tracheal washings, lachrymal fluid and saliva have been found to be not necessarily the same as those in the serum following IBV infection [5]. Thus, it would be valuable to titrate such fluids from IBV-infected chicks for their antibody to IBV.

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