Comparison of the Susceptibility of Chicks of Different Ages to Infection with Nephrosis / Nephritis-Causing Strain of Infectious Bronchitis Virus

Samuel Baltazar ANIMAS, Koichi OTSUKI*, Misao TSUBOKURA***, and Jane K. A. COOK**
Departments of Public Health, Microbiology, Faculty of Agriculture, Tottori University, Tottori 680, Japan, and AFRC Institute for Animal Health, Houghton Laboratory, Houghton, Huntingdon, Cambridgeshire, PE17 2DA, England

(Received 29 November 1993/Accepted 6 December 1993)

ABSTRACT. Two- and 6-week-old chicks were inoculated with the Kagoshima-34 strain of avian infectious bronchitis virus. Serum, bile, Harderian gland, lacrymal fluid, saliva and tracheal washings were collected and their antibody content determined using neutralisation tests. The neutralising antibody (NA) in the serum and bile was detected earlier and in slightly higher concentration in the 6-week-old chicks. Although there was no marked difference in the levels of NA in other body fluids, it was detected earlier in the 6-week-old chicks. In both experiments, the clinical signs were more severe in the 2-week-old chicks. Recovery of virus from the trachea of both ages was not different but virus was recovered for longer in the lungs, kidneys and colon of the 2-week-old chicks. This is the first report wherein IBV-neutralising antibody in the bile is described.—Key words: age, antibody production, avian infectious bronchitis, body secretion, virus recovery.

Avian infectious bronchitis (IB) is widespread and of economic importance wherever poultry are produced. Although it has been the subject of many studies for more than 60 years, the factor(s) involved in the susceptibility or resistance of chickens to the infection have not been fully resolved.

It has been reported that systemic and local immune responses are essential for the adequate protection of chickens against IBV [9]. Studies on these aspects of IBV infection have already been reported in different breeds [15] or inbred lines [7, 13, 14] of chicken. However, there appears to be no such reports in chicks of different ages. In a previous report [4], it was found that younger chicks were more susceptible than older ones to IBV infection. However, the number of experimental birds used was limited and thus, the variation of individual response to the infection was not well determined. In addition, the local immune response of infected chicks was not investigated.

The present investigation was therefore undertaken to further elucidate the differing susceptibility of chicks of different ages to infection with IBV on the basis of clinical signs, virus recovery from selected organs and neutralising antibody (NA) production in serum, tracheal washings, Harderian gland (HG), and body fluids.

MATERIALS AND METHODS

Virus: The Kagoshima-34 (K-34) strain of IBV used was described previously [4] and titrated in tracheal organ cultures (OC) as described by Cook et al. [6].

Chicks and embryonated eggs: Specific-pathogen-free (SPF) chicks and embryonated eggs of a White leghorn line were kindly supplied by Dr. Y. Iritani, Aburahi Laboratories, Shionogi Company, Japan. Experiment 1: Fifty one 2-week-old and 51 6-week-old chicks were inoculated intratracheally with 0.1 ml of K-34 with a titre of 10^5.5 median ciliostatic dose (CD50). Samples were collected from 5 birds at 1 week interval for 6 weeks, and from 3 birds every 2 weeks thereafter until 20 weeks post inoculation (PI). Serum, bile, HG, tracheal washings, lacrymal fluid and saliva were examined for NA response whilst the rest of the organs were collected for virus recovery.

The hepatic bile was collected using the surgical procedure described by Rose et al. [17]. Briefly, the chicken was laid on its back, right side towards the operator and an incision of 2-3 cm long was made posterior to the last rib. By applying gentle traction, that part of the duodenal loop which lies immediately beneath the incision was brought to the exterior, exposing 2 bile ducts with the adjacent part of the pancreas. After being freed by blunt dissection, a small incision was made in the hepatopancreatic duct and the collecting tube was inserted, tied in place and the bile allowed to flow to a small bottle attached to the tube. This surgery was carried out while the chicken was under inhalant anesthesia (Ethrane (R)).

After 24 hr, bile samples were harvested, and the collection of the rest of samples followed. Blood was taken usually from the wing vein, but for young chicks, by cardiac puncture. Pooled samples of lacrymal fluid, and pooled samples of saliva were collected following injection of carbamylcholine chloride at a dose of 0.7 mg/kg body weight [1]. Chicks were then sacrificed and pooled samples of the HG, trachea, lungs, kidneys and colon were aseptically removed. The tracheal washings were obtained individually by aspiration, as described previous-
ly [12]. Prior to test, all samples were stored in -35°C.

**Experiment 2:** Expt. 2 was performed exactly as Expt. 1; using the same number of experimental birds of both ages, and the same virus and virus dosage, but it was performed at a different time. This was done for the purpose of comparison and confirmation of the results of Expt. 1.

**Neutralisation test:** The chicken sera, HG, tracheal washings and all body secretions were tested for NA to IBV using OC as described by Cook et al. [6]. Titres of all samples were calculated by the method of Reed and Muench [16].

**Virus recovery:** Virus recovery was attempted by applying the same procedure as used previously [4]. However, in this investigation, samples were passaged only once in SPF embryonated eggs.

**RESULTS**

**Clinical signs:** In both experiments, the 2-week-old chicks showed the same clinical signs as those observed previously [4], i.e. the respiratory signs were more severe and diarrhoea lasted longer in the 2-week-old than in the 6-week-old chicks.

**Antibody in serum:** Figure 1(a) shows the profile of serum NA production in Expt. 1. The NA in the 2-week-old chicks was detected from 6 weeks PI and continued up to at least 20 weeks PI with peak antibody titre at 14 weeks PI (Geometric mean neutralising antibody titre [GMT]=2.5). In the 6-week-old chicks, the NA was detected earlier (at 3 weeks PI) and also continued throughout this investigation, producing a peak antibody level at 20 weeks PI (GMT=2.7). The profile of GMT shown in Fig. 1(a) indicates the difference in the antibody response between the 2 ages. In Expt. 2, the NA in 6-week-old chicks was higher only from 3 to 5 weeks PI, and after that, NA in both ages was not statistically different (Fig. 1[b]).

**Antibody in the bile:** As shown in Fig. 2(a), NA in the bile of both ages was detected from 2 weeks PI and continued until at least 20 weeks PI. However, the titre in the 6-week-old chicks was higher (GMT=2.1 to 2.8) compared to that in the 2-week-old chicks (GMT=1.4 to 2.5).

In Expt. 2 (Fig. 2[b]), a slightly higher titre of NA was detected at the first 2 weeks PI in the bile of 6-week-old chicks, and after that, a similar titre of NA was detected in this fluid of both 2- and 6-week-old chicks (peak GMT=2.1 to 2.9).

**Antibody in the Harderian gland:** Figure 3 shows the results of determining neutralising antibody titres in the HG. Neutralising antibody in the 6-week-old chicks, although lower (Neutralisation index [NI]=<1.0 to 1.9) particularly in Expt. 1, was detected earlier (2 weeks PI) than in the 2-week-old birds. However, the 2-week-old chicks produced a higher titre of NA (NI=<1.0 to 2.5) which lasted until at least 20 weeks PI.

**Antibody in the Saliva:** In Expt. 1, NA was detected at 3 weeks PI in the 6-week-old chicks, and at 5 weeks PI in the
2-week-old ones (Fig. 4[a]). In Expt. 2 however, the NA in the 2-week-old chicks was detected earlier (at 4 weeks PI) than in the 6-week-old ones, where it was first detected at 6 weeks PI (Fig. 4[b]). Titres in the two ages were not statistically different.

Antibody in the lachrymal fluid: Neutralising antibody was detected at almost the same time (3 weeks PI) in both ages (Fig. 5). In Expt. 1 (Fig. 5[a]), the antibody titre in the fluid of the 2-week-old chicks was consistently low from 3 until 12 weeks PI. Thereafter, antibody was not detected. In the 6-week-old chicks, NA was only detected at 5, 14 and 20 weeks PI. In Expt. 2, the antibody titre in both ages was similar (NI≤<1.0 to 2.0), and again titres were low.

Antibody in the tracheal washings: The profile of NA production in the tracheal washings in both experiments are shown in Fig. 6(a) and (b). Virtually no antibody was detected in chicks of either age throughout the period of the experiments.

Virus recovery: The results of attempted virus recovery from the organs of infected chicks are shown in Table 1. In Expt. 1, virus was recovered from the kidneys and lungs of both ages for the first 3 to 4 weeks PI. However, recovery from the 2-week-old chicks continued for longer; until 10 weeks PI in the lungs and 4 weeks PI in the kidneys. Virus was recovered from the trachea, only at 1 week PI in the 2-week-old chicks whilst in the 6-week-old ones, it was only recovered at 2 weeks PI. No virus was recovered from the colon of both ages in this experiment. In Expt. 2, virus was recovered from the lungs and kidneys of both...
Table 1. Recovery of IBV strain K-34 from different organs of infected chicks

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>1</th>
<th>2</th>
<th>1</th>
<th>2</th>
<th>1</th>
<th>2</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ</td>
<td>Lung</td>
<td>Kidney</td>
<td>Trachea</td>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week(s) PI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>+/</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>2</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>3</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>4</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>5</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>6</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>7</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>8</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>9</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>10</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>11</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>12</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>13</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>14</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>15</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
</tbody>
</table>

+ : Virus recovered.
- : No virus recovered.
+-: Not confirmed.
Expts. 1 and 2: 2-week-old/6-week-old chicks.

ages for the first 2 weeks PI. However, at 6 and 8 weeks PI, virus was again recovered from the kidneys of 2-week-old chicks. No virus was recovered from the trachea of both ages. IBV was recovered from the colon at 2 to 4 weeks PI in the 2-week-old chicks, but this was not confirmed since recovery was attempted only once in ovo. At 12 and 18 weeks PI, virus was again recovered from the colon of the 2-week-old chicks. No virus was recovered from the colon of the 6-week-old chicks.

**Discussion**

In the previous report [4], it had been demonstrated that the serum NA was produced earlier and in higher concentration in the 6-week-old chicks than in the 2-week-old ones, although the latter showed more severe clinical signs. This result seems to be confirmed in the present investigation. Although the profile of production of IBV-serum-NA in the 2-week-old chicks was similar to that in the previous report, the NA in the present investigation lasted longer. In the previous report, only 4 chicks were infected with IBV and these were bled repeatedly throughout the period of the experiment, whilst in the present investigation a total of 51 chicks were inoculated and 3 or 5 chicks were killed for every sampling. The 2-week-old chicks perhaps may be too young to tolerate the severe stress caused by repeated bleeding. The chicks tested in the previous study have been subjected to stress several times and this perhaps explains why the antibody production of the 2-week-old chicks was lower than reported in this study.

It is interesting to note that a higher titre of NA was detected in hepatic bile than in serum. This findings has been reported also in the case of avian coccidiosis [11]. In both ages, NA in the bile was detected earlier, present at a higher titre and lasted longer than in the serum.

It has been reported that IBV can be consistently recovered from the faeces of IBV-infected chicks for a long period after experimental infection [2, 3]. The isolation from a field case of an IBV having enterotropism has also been reported [10]. These reports therefore indicate that the epithelial cells of the intestine are undoubtedly one of the targets for IBV. The presence of a high concentration of NA in the bile in both ages, may play an important role in protection against the infection by neutralising the virus in the gut and consequently preventing further infection of other tissues.

These results suggest that even when NA against IBV can not be detected in serum, it is possible that it will be detected in the bile. In the future, the bile could be one of the best samples to use to investigate IBV immunologically. When bile is titrated for antibody using some immunological tests, samples from the hepatic bile may be more satisfactory since it is less viscous than the cystic bile and a larger volume could be collected.

Baba et al. [5] reported that immunoglobulins in the lachrymal fluid originate from the HG. It is thus, surprising that the profile of NA production in the HG of chicks in both ages did not exactly relate to that in the lachrymal fluid. The NA production in the HG of the 2-week-old chicks was slightly higher than that of the 6-week-old one although it was detected earlier in the latter. This seems to suggest that the level of the NA antibody response against IBV in one target tissue may not necessarily be the same as that in other tissues. The higher NA production in the HG of the 2-week-old chicks is poorly understood and requires further investigation.

Only low levels of NA were detected in the tracheal washings of the 2- and 6-week-old chicks and with no significant differences between ages. The M-41 strain of
IBV induced considerable titre of antibody in the tracheal washings of infected chicks [7, 13, 14]. Thus, it is unexpected that the IBV strain K-34 did not induce a high level of NA in the tracheal washings since this virus, caused not only nephritis/nephrosis in chickens but, like M-41, respiratory disease as well. Some IBV strains causing nephrosis/nephritis should be investigated to confirm this result.

It has been reported that the mechanism(s) involved in the more rapid recovery from IB infection of resistant inbred line chicks appear to operate within the first few days of inoculation [14]. In these experiments, although there was no significant difference in the level of production of NA in either age, the earlier production of antibody, which was observed consistently from all tested samples, might play a role in inhibiting further virus growth and tissue damage in the 6-week-old chicks. Although there could be several factors involved, this perhaps, in part, may account for the milder clinical signs observed in the older chicks.

The results of attempted virus recovery from the trachea in both ages were similar. However, the virus was recovered for longer from the lungs, kidneys and colon in the 2-week-old chicks. This may not give a complete picture of the accuracy of virus recovery however, since the previous study [4] showed that it took at least 3 passages in ovo before virus were recovered from some samples. Nevertheless, the persistence of virus in the 2-week-old chicks was again demonstrated.

Cronion and Hofstad [8] reported that chickens infected with IBV at very young age tend to become non-egg layers. It would have been interesting if chicks could have continued to be observed until they started to lay eggs so that the effect on egg production of infected with IBV at both ages could have been investigated.

Although the experimental infections with IBV were performed twice under the same conditions, the results obtained were not necessarily the same. Cook et al. [7] showed a similar phenomenon. An explanation for the inconsistent results obtained in this investigation is not immediately forthcoming. This inconsistency is probably one of the special characters of IBV, especially field isolates which makes it difficult to analyse. Thus, it is very difficult to conclude whether or not the local immune secretions indeed play an important role in the differing susceptibility of chicks of different ages to IBV infection. However, since there was no significant difference in the levels of NA among body fluids found in this investigation, it is most likely that other factors are involved. Further study is necessary to confirm these results.

REFERENCES