# Pathogenicity of Newly Isolated Picornavirus (Avian Nephritis Virus) for Chicken

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A picornavirus, immunologically distinct from avian encephalomyelitis virus (AEV), was isolated from the rectal contents of apparently normal broiler chicks in chicken kidney cell (CKC) cultures when virological examination was carried out to clarify the infections of avian viruses on field broiler chicken flocks in 1976.[8] It was later demonstrated that the virus causes nephritis and retarded young chicks.[1,6] In order to distinguish the virus from AEV, the virus was termed avian nephritis virus (ANV).

This report deals with characteristics of the virus,[8] pathogenicity for chicken and chick embryo in experimental inoculation,[1,3,4,5] and epidemiology of the virus.[2]

## Biological and physicochemical properties of the virus

The physicochemical and biological properties of ANV are very similar to AEV, but the virus is distinct from AEV serologically and pathologically.

ANV has the following properties: 1) the presence of RNA; 2) growth in the cytoplasm (Plate 1); 3) 28 nm in diameter (Plate 2); 4) resistance to ethyl ether, chloroform, trypsin, and acid; 5) relative heat lability; 6) partial stabilization at 50°C by molar magnesium chloride. The density in CsCl of the complete virion was not estimated because the virus is very unstable in a strong solution of CsCl.

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ANV grew well in CKC (Fig. 1) with the round type cytopathic effect (CPE), but did not grow in duck embryo fibroblasts, duck embryo kidney cells, and some established cell lines (Hela, Vero, MDBK, PK 15, and MDCK).

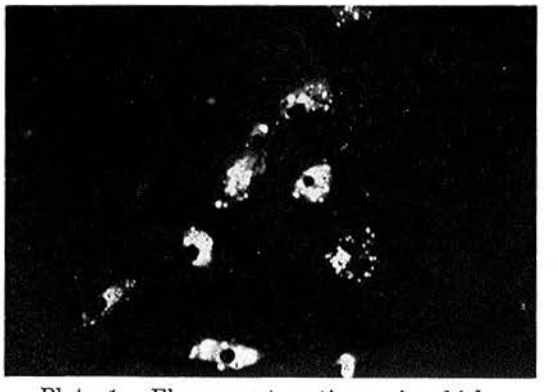

Plate 1. Fluorescent antigens in chicken kidney cells infected with ANV Lumpy and granular antigens are seen in the cytoplasm ×300

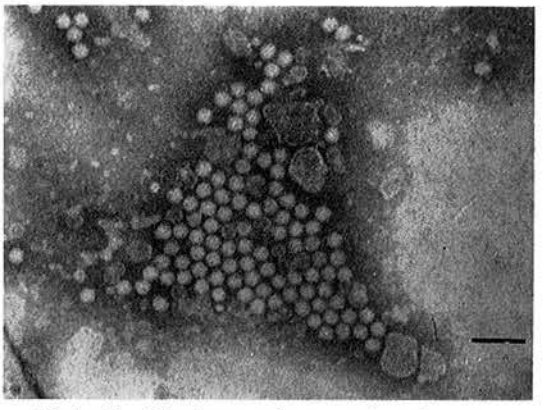

Plate 2. Electron micrograph of negatively stained ANV particles Bar=100 nm

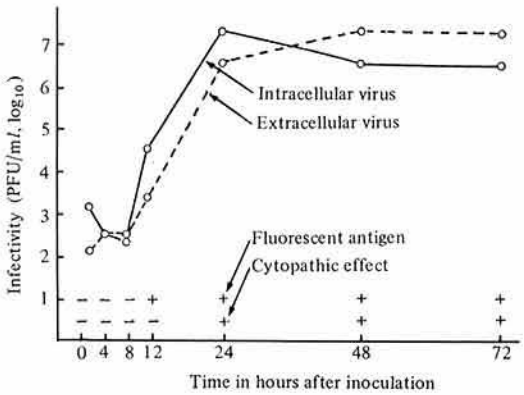


Fig. 1. Growth curves and appearance time of fluorescent antigen and cytopathic effect of ANV in chicken kidney cell culture

## Pathogenicity and distribution of ANV in inoculated one-day-old chicks

The pathogenicity of ANV for 1-day-old chicks was studied by intraperitoneal inoculation. No clinical signs were observed, but mean body weight was significantly lower ($P < 0.01$) in inoculated groups than in control groups 7 days post-inoculation (PI) (Fig. 2). A mild yellowish discoloration of the kidneys was noticed at necropsy 7 to 21 days PI (Plate 3). In a chronological study on the distribution of the virus in organs, the virus was consistently isolated from kidney, jejunum, and rectum of infected chicks but not from brain and trachea during the first 10 days PI. After that time the virus apparently disappeared (Table 1). Specific immunofluorescent antigens were seen predominantly in the epithelia of the renal tubules during the first 7 days PI (Plate 4).

## Pathological changes in chicks inoculated with ANV

Distinct gross and microscopic lesions were observed only in kidneys of experimentally infected chicks. Histologically, focal lesions were observed in the cortex of kidneys from 3 to 21 days PI. The primary change was

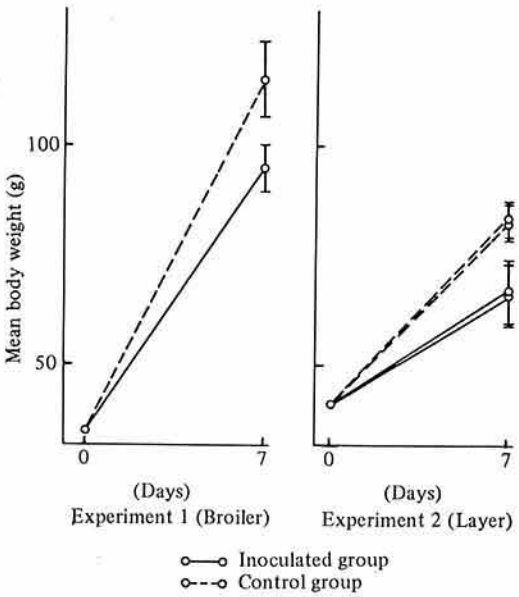


Fig. 2. Effect of ANV on increase in body weight

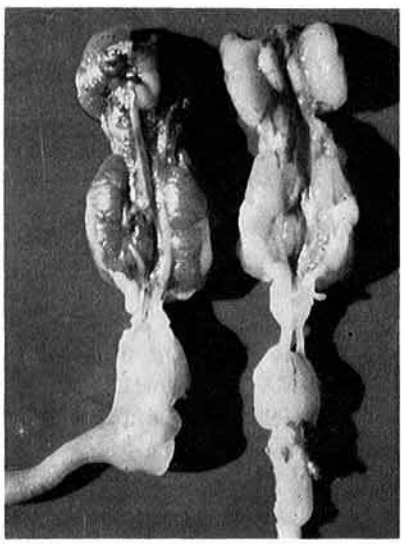

Plate 3. Gross lesions in the kidneys
7 days PI. Left: control, Right: infected. Yellowish discoloration is present in the infected kidneys.

**Table 1. Distribution of the virus in organs of broiler chicks inoculated intraperitoneally with ANV**

| Days after inoculation | 1 | | 2 | | 3 | | 4 | | 5 | | 8 | | 10 | | 14 | | 21 | | 28 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Brain | — | —[a] | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Trachea | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Thymus | — | — | — | — | — | 2.4[b] | — | — | — | 1.9 | — | — | — | — | — | — | — | — | — | — |
| Lungs | — | — | — | — | — | 1.7 | — | — | 2.9 | — | — | — | — | — | — | — | — | — | — | — |
| Liver | — | — | 2.1 | 2.7 | — | 4.1 | 1.4 | — | 1.4 | — | 1.4 | — | — | — | — | — | — | — | — | — |
| Spleen | — | 1.4 | — | 3.4 | 2.9 | 3.4 | 3.7 | 3.5 | 3.9 | 2.4 | — | — | — | — | — | — | — | — | — | — |
| Pancreas | — | — | — | — | — | 2.9 | — | 2.7 | 1.7 | — | — | — | — | — | — | — | — | — | — | — |
| Kidneys | — | — | 3.0 | 3.7 | 4.9 | 4.9 | 4.5 | 6.2 | 5.7 | 4.1 | 4.0 | 4.4 | — | 3.1 | — | — | — | — | — | — |
| Jejunum | — | — | — | 4.1 | 5.4 | 5.7 | 5.3 | 4.8 | 4.6 | 5.5 | 4.0 | 3.9 | — | 4.5 | — | — | — | — | — | — |
| Rectum | — | — | 3.3 | 3.6 | 4.9 | 4.7 | 3.4 | 4.5 | 5.0 | 4.7 | 4.5 | 4.5 | — | 3.9 | — | — | — | — | — | — |
| Bursa | — | 2.4 | — | 3.1 | 4.2 | 3.9 | 3.4 | 4.5 | 5.0 | 3.0 | 3.2 | 2.4 | — | — | — | — | — | — | — | — |
| Antibody[c] | <4 | <4 | <4 | <4 | <4 | <4 | <4 | <4 | <4 | <4 | 4 | 4 | 8 | 4 | 32 | 64 | 32 | 32 | 16 | 8 |
| Gross lesion in kidneys[d] | N | N | N | N | N | N | N | N | N | N | P | P | P | P | P | P | P | N | N | N |

a) Negative in 10% suspension of the organ.
b) Log-PFU per m*l* of 10% suspension of the organ.
c) The figure indicates the reciprocal of the serum dilution positive for fluorescent antibody.
d) N and P for lesions: Negative and positive for lesions, respectively.

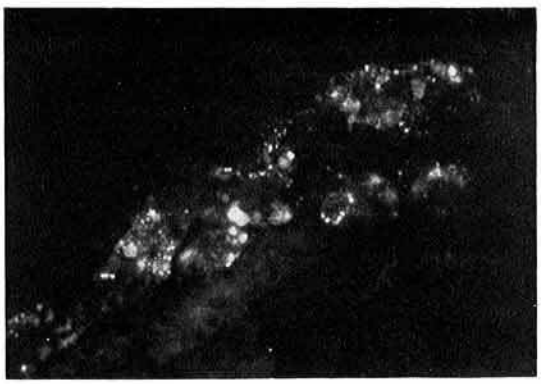

Plate 4. Immunofluorescent staining of ANV antigens in the epithelia of renal tubules
3 days PI. ×200

degeneration of epithelial cells of the proximal convoluted tubules with infiltration of granulocytes. The degenerating epithelial cells contained acidophilic granules of various sizes in their cytoplasm (Plate 5). The lesion was accompanied by interstitial lymphocyte infiltration and moderate fibrosis. In the later stages 21 to 28 days PI, lymphoid follicles developed. ANV particles and viral antigens were demonstrated in the degenerating epithelium both in electron microscopic (Plate 6) and immunofluorescent studies.

## Susceptibility of chickens to ANV by inoculation routes and ages

Factors that influence the response of chickens to ANV were studied. Day-old specific-pathogen-free (SPF) chicks were inoculated with ANV by oral, subcutaneous, intratracheal, intramuscular, and intracerebral routes at a dose of $10^{5.0}$ plaque-forming units (PFU) per chick. Inoculation of all routes induced only nephritis (Table 2), and contact infection of ANV occurred very easily.

When 1-, 14-, 28-, 56-, and 300-day-old chickens were inoculated orally with $10^{5.0}$ PFU of the virus, the day-old chicks appeared to

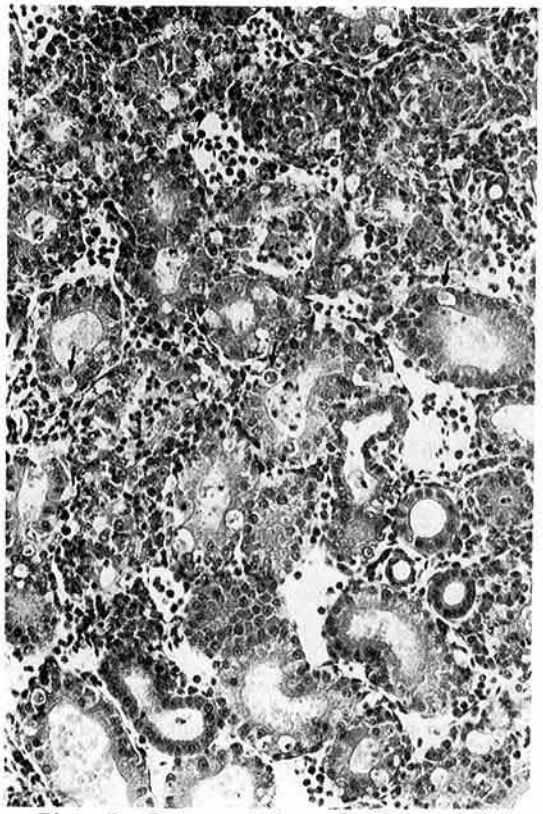

Plate 5. Degenerated proximal convoluted tubules containing acidophilic granules (arrows) in epithelial cytoplasm, and lymphocytic infiltration in interstitium
5 days PI. Hematoxylin and eosin staining. ×400

Plate 6. Crystalline array of virus particles in the cytoplasm of a kidney epithelial cell
3 days PI. ×30,000

**Table 2. Comparison of susceptibility to ANV of day-old chicks 7 days PI via various routes**

| Routes | No. inoculated | No. with gross lesion in kidney | No. with nephritis | No. with fluorescent antigen in kidney |
|---|---|---|---|---|
| Oral | 10 | 8 | 10 | 8 |
| Control | 5 | 0 | 0 | 0 |
| Subcutaneous | 10(1)[a)] | 7 | 9 | 4 |
| Control | 5 | 0 | 0 | 0 |
| Intratracheal | 10(1) | 8 | 9 | 5 |
| Control | 5 | 0 | 0 | 0 |
| Intramuscular | 10 | 9 | 10 | 4 |
| Control | 5 | 0 | 0 | 0 |
| Intracerebral | 10 | 8 | 10 | 4 |
| Control | 5 | 0 | 0 | 0 |

a) The figure in parentheses represents number of accidentally dead chicks.

be most susceptible; histopathological responses to the virus became weak as chicks aged. The serological response of 28-day-old chickens to ANV was stronger than those of other groups. Pathologically, the adult chickens hardly responded to the virus, but they produced antibodies against the virus (Table 3).

## Distribution of ANV in inoculated laying hens

The pathogenicity of ANV for SPF laying hens was studied by the intravenous route of inoculation. The distribution of the virus in organs, histological changes in main organs,

**Table 3. Effect of age on susceptibility of chickens to ANV**

| Age at inoculation (days) | Antibody titer in preserum | 7 days PI | | | | 14 days PI | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. inoculated | No. with gross lesion in kidney | No. with nephritis | Antibody titer in serum | No. inoculated | No. with gross lesion in kidney | No. with nephritis | Antibody titer in serum |
| 1 | <4 | 4 | 4 | 4 | <4 | 3(1)[a] | 0 | 3 | 51 |
| 14 | <4 | 4 | 2 | 4 | 3[b] | 4 | 0 | 4 | 91 |
| 28 | <4 | 4 | 0 | 4 | 3 | 4 | 0 | 4 | 151 |
| 56 | <4 | 4 | 0 | 1 | <4 | 4 | 0 | 3 | 27 |
| 300 | <4 | 4 | 0 | 1 | <4 | 4 | 0 | 0 | 11 |

a) The figure in parentheses represents member of accidentally dead chicks.
b) The figure indicates a geometric mean titer calculated by the reciprocal of the highest dilution positive for fluorescent antibody.

**Table 4. Distribution of the virus in organs and development of antibody in sera of hens inoculated intravenously with ANV**

| Days after inoculation | Inoculated group | | | | | | | | | | | | | | Control group | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | 4 | | 6 | | 8 | | 10 | | 14 | | 27 | | 0 | | 6 | | 27 | |
| Chick No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 18 | 19 | 20 | 21 | 22 | 23 |
| Brain | —[a] | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Trachea | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Lung | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Liver | 3.6[b] | 3.0 | 1.6 | 2.3 | 2.4 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Spleen | 2.8 | 3.2 | 1.2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Pancreas | 0.4 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Kidney | — | 1.0 | — | 2.7 | 1.2 | 3.4 | 1.1 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Jejunum | 2.6 | 3.1 | 2.5 | 3.2 | 3.0 | 4.1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Rectum | — | 2.6 | 0.7 | 2.4 | 2.0 | 2.5 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Ovary | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Oviduct | | | | | | | | | | | | | | | | | | | | |
| Mugnum | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Isthmus | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Uterus | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Serum | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Antibody[c] | <4 | <4 | <4 | <4 | <4 | <4 | <4 | <4 | 8 | 8 | 4 | 4 | 4 | 16 | <4 | <4 | <4 | <4 | <4 | <4 |

a) Negative in 10% suspension of the organ.
b) Log-PFU per ml of 10% suspension of the organ.
c) The figure is the reciprocal of the highest serum dilution positive for indirect fluorescent antibody method.

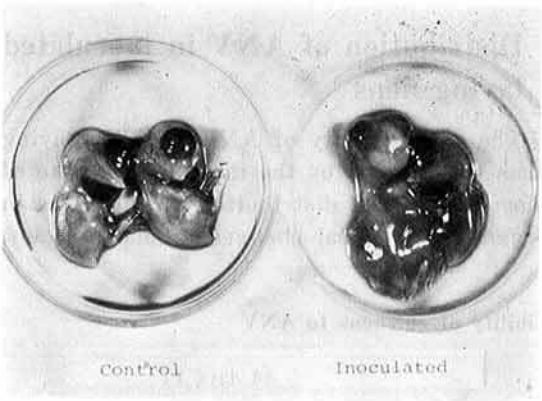


Plate 7. Infected 10-day-old embryos showing hemorrhage and edema of whole body
4 days PI by the yolk sac route.

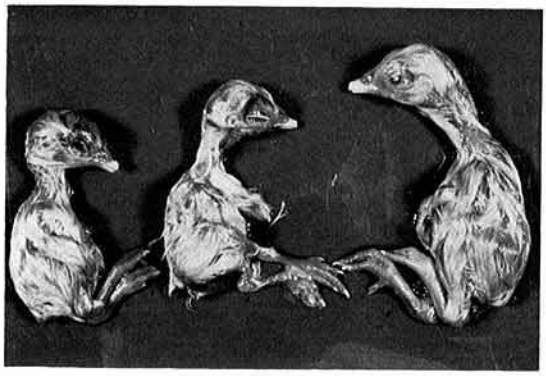

Plate 8. Two infected 20-day-old embryos showing stunting
14 days PI by the yolk sac route, Right: control.

**Table 5. Egg production rate (hen-day basis) during 4 weeks before and after virus inoculation**

| Group | No. of hens | First egg laying (Age in days) | Egg production rate (%) (hen-day basis) | |
|---|---|---|---|---|
| | | | Before inoculation | After inoculation |
| Inoculated group | 17 | 168.7±14.0[A)] | 75.1± 7.0 | 86.0±6.0 |
| Control group | 6 | 175.5±20.3 | 67.6±17.9 | 87.5±7.5 |

A) Mean ± standard deviation.

the condition of laying, and egg transmission of the virus were examined. Over an experimental period of 27 days, no clinical signs were observed. In a chronological study on the distribution of the virus in organs, the virus was recovered from liver, kidney, jejunum, and rectum for 6 days PI. The virus titer in organ emulsion was the highest in the jejunum of all the main organs. The virus was recovered from the kidney for 8 days PI, although the titer was not so high in this organ. It was not recovered from the ovary or oviduct (Table 4). In a pathological examination, some local inflammatory changes were observed only in kidney. There were no significant changes in the ovary, oviduct, or any other organ. Antibody appeared 10 days PI and was detectable even 27 days PI, although it was not so high in titer. There was no significant difference in the rate of egg production between the infected and the sham inoculated groups (Table 5). No virus was isolated from 111 fertile eggs laid by infected hens over a period from 2 to 27 days PI.

## Pathogenicity of ANV for embryonating hen's eggs

The pathogenicity of ANV for embryonating hen's eggs was studied by various routes of inoculation. When inoculated with ANV by the yolk sac route, 6-day-old embryos showed the highest susceptibility and all of them died 3 to 14 days PI. They manifested hemorrhage and edema of the whole body (3 to 6 days PI) (Plate 7) and stunting (7 to 14 days PI) (Plate 8). The 50% egg-infective dose of the virus by yolk sac inoculation

coincided well with the virus titer expressed in PFU determined on the monolayer of CKC (Table 6). The virus could be passed serially through the chorioallantoic membrane (CAM) of embryonating hen's eggs. In these eggs the CAM showed edematous thickening at the inoculation site, and the embryo stunting. When inoculated by the CAM route, high virus doses killed all embryos, but low virus doses allowed some of infected embryos to hatch normally. When inoculated by the allantoic cavity route, the virus did not multiply in the allantoic cavity of embryonating eggs, but some of these eggs became infected. Fluorescent antigens were present only in the kidneys and the CAM of embryos infected with the virus. The virus was recovered at a low rate from cloacal swabs of chicks from normally hatched eggs inoculated with the virus by the CAM route (Table 7). These chicks were variable in growth, but had antibodies against the virus and developed nephritis at 36 days of age.

**Table 6. Comparison of infective titers of ANV in chicken kidney cell cultures and in embryonating eggs by yolk sac inoculation**

| $EID_{50}$ per m*l* ($log_{10}$) | PFUs per m*l* ($log_{10}$) |
|---|---|
| 6.7 | 6.0 |
| 6.1 | 5.8 |
| 6.5 | 6.1 |

**Table 7. Virus isolation from cloacal swabs of chicks normally hatched from eggs inoculated with ANV**

| Virus dilution | Number of positive/ Number of tested |
|---|---|
| $10^{-1}$ | 2/2 |
| $10^{-2}$ | 1/3 |
| $10^{-3}$ | 1/2 |
| $10^{-4}$ | 0/3 |
| $10^{-5}$ | 2/8 |
| Control | 0/10 |

Inoculation into embryonating eggs: 10-day-old embryonating eggs were inoculated onto CAM with $10^{-1}$~$10^{-5}$ dilutions of virus ($10^{4.8}$ PFUs/0.1 m*l*).

## Epizootiology

Of 916 chicken sera collected from 99 flocks submitted for diagnosis to the author's laboratory from 1972 to 1978, 99 sera from 43 layer and broiler flocks were positive for antibody when examined by the indirect fluorescent antibody test. In this test antibody began to be detected in 1973. Over a period from 1975 to 1978, the rate of positive flocks in the tested flocks ranged from 35 to 62%, although the rate among samples ranged from 12 to 17%. There were 9 flocks in which the positive rate exceeded 50%. There was no difference in the rate between layer and broiler chickens. In addition, viruses which were identified as ANV by immunofluorescence were isolated from 10 chicken flocks which were not regarded as the antibody positive flocks. It is thus presumed that ANV is prevalent among commercial chicken flocks in Japan.

## Conclusions

As there have been no reports of disease associated with ANV infection in the field, its geographic distribution, incidence and economic consequences are not known. However nephritis is a common necropsy finding in birds, and the possibility for an etiological role of ANV needs investigation. In infected flocks in Japan the infectivity rate is very high, approaching 100%. Certain nephrotoxic strains of infectious bronchitis virus (IBV) cause interstitial nephritis. It would be difficult to separate the two conditions on the basis of the histological lesions.[7] These cases may be differentiated from ANV infections by the fact that there are some changes in the trachea and usually IBV infections in kidneys are preceded by respiratory signs. When a nephritis is diagnosed, it is necessary to isolate the causative agent or to determine the antibody titer.

Chickens of all ages may be infected, but it has been observed that 1-day-old chicks are the most susceptible to ANV. Transmission readily occurs by direct or indirect contact. It is obscure whether this virus transmits

via eggs or whether this virus infection can exacerbate any other disease. It is known, however, that experimental infection with ANV causes nephritis and retarded young chicks. So the role of this virus in complicated infections of commercial young chicken flocks may be worthy of close observation.

## References

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