A Survey of Chicken Sera for Antibody to Atypical Avian Rotavirus of Duck Origin, in Japan
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Serological evidence of rotavirus infection in avian species has been reported [1, 2, 7, 10, 12–15, 17]. And, many rotaviruses have also been isolated from chickens, turkeys, pheasants, pigeons, and lovebirds [3, 4, 6, 8–10, 12, 15, 16, 18]. Some avian rotaviruses isolated were shown to lack a common group antigen demonstrable by immunofluorescence (IF) test or to have a different electrophoretic pattern of RNA segments from conventional rotaviruses, and these were tentatively referred to as atypical rotaviruses [2, 5, 7–9, 11].

In Japan, however, only a few works have been done on rotavirus infection in avian species [12, 14, 15]. Sato et al. [14] and Minamoto et al. [12] reported the prevalence of antibody to rotavirus in chicken sera, using conventional rotaviruses of bovine or pigeon origin, respectively. Meanwhile, the present authors [15] have isolated atypical rotaviruses, which did not cross-react with the conventional rotaviruses by IF test, from duck feces. A representative isolate of them was designated F-29 strain.

This paper describes the antibody production of chickens infected with the F-29 strain experimentally, and a survey of antibody to the virus in chicken flocks.

Firstly, fifteen 7-day-old chicks obtained from a specific pathogen-free (SPF) chicken flocks were inoculated orally with $10^5.0$ fifty percent tissue culture infective dose of the F-29 strain at 19th passage level in chicken kidney cell (CKC) cultures per bird. They were reared in an isolated cage in contact with three non-treated chicks of the same day-old. Ten control chicks were reared in an another isolated cage. After administration of the virus, two or three birds from virus-inoculated and control groups were bled every week, respectively, for 4 weeks postinoculation (PI). At the last week, three contact birds were also bled.

The sera were examined for antibody titers to the F-29 strain in two-fold dilutions in physiological buffered saline (PBS) beginning with a 1:10 dilution by indirect IF test as follows. Monolayers of CKC cultures grown on cover-slips (18×18 mm) in petri dishes were infected with the F-29 strain, incubated at 37°C for 18 hr, fixed in acetone at room temperature for 5 min, then air-dried and stored at 4°C until use. The fixed cell cultures on cover-slips were divided into 6 areas with nail-enamel. These areas were covered with test sera and incubated at 37°C for 1 hr. Following a wash in PBS, counter staining was performed for 1 hr with the anti-chicken IgG rabbit serum conjugated with fluorescein isothiocyanate. After a further wash in PBS, cover-slips were mounted in buffered glycerol and examined microscopically for specific fluorescence. The IF antibody titer was expressed as the reciprocal of the highest serum dilution showing obvious fluorescence.

All chicks employed in this study showed no clinical signs throughout the observation period.

Antibody titers are shown in Fig. 1. Seroconversion was observed in almost all inoculated chicks from 2 weeks PI and contacted birds at 4 weeks PI, but not in any control birds. Antibody titers reached 1:40.

These results show that chicks have higher susceptibility to the F-29 strain, and the virus

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Fig. 1. Detection of antibody to the F-29 strain of avian rotavirus by indirect immunofluorescence test in chicks (○: inoculated, ●: contacted, □: control) after oral inoculation with $10^5.0$ fifty percent tissue culture infective dose of the F-29 strain per bird at 7-day-old.
could be transmitted horizontally among pen-mates.

Secondly, a survey of antibody to the F-29 strain was carried out using 1,040 chicken sera collected from 208 chicken flocks reared in 25 prefectures during two years from 1988 to 1989. Five sera were tested per flock. The sera were diluted 1:10 in PBS and use in indirect IF test mentioned above.

Antibody was detected in 83 (91.2%) of 91 broiler and in 101 (86.3%) of 117 layer flocks. The positive rates among samples grouped by four districts (Hokkaido-Tohoku, Kantoh-Chubu, Kinki-Chusikoku, and Kyushu) are shown in Table 1. The positive rates ranged from 54.7 to 70.9% in broilers and from 44.0 to 58.6% in layers. The highest rate was obtained in Kyushu district and the lowest was in Hokkaido-Tohoku district. Also, broiler was about 10% higher than layers in the positive rate in each district.

Table 2 shows the prevalence of antibody in sera grouped by different ages. At younger age less than 40-day-old, antibody was detected in 80 (59.3%) of 135 sera tested. Meanwhile, at older age more than 401-day-old, it was also detected in 32 (64.0%) of 50 sera tested. These suggest that rotavirus infection may occur quite early in the life of chickens. However, it is not clear whether the antibody detected at 401-day-old or older age is that of a continual state followed by first infection at younger age or that of produced by reinfection at older age.

Out of 1,040 sera tested in this study, 200 sera were collected from 40 breeder flocks. Of these flocks, antibody was detected in 117 (58.5%) sera from 35 (87.5%) flocks. Presumably, rotavirus may be prevalent among breeder flocks as in commercial flocks.

Additionally, antibody titers were examined in 25 sera from five flocks selected for the reason that antibody was detected in all five sera of each flock tested. Their titers ranged from 1:10 to 1:640 with a geometric mean titer of 1:80.

The present study shows that atypical rotavirus-uses are widespread in chicken flocks in Japan.

In the previous works reported by Sato et al. [14] and Minamoto et al. [12], antibodies to conventional rotavirususes of bovine or pigeon origin were detected in 56.0 or 57.1% of chicken sera tested by neutralization or hemagglutination inhibition tests, respectively. Our data presented in this study were very close to those of them. Therefore, it is assumed that infections with conventional and atypical rotavirus-uses may occur with similar frequency in chicken flocks in Japan. McNulty et al. [7] have also reported similar findings in Northern Ireland.

**REFERENCES**

SEROSURVEY FOR ROTAVIRUS IN CHICKENS


要　約

鶏群のアヒル由来非定型ロタウイルスに対する血清疫学調査（文報告）：高瀬公三・内村哲也・香月伸彦・山元通孝（財）化学及血清療法研究所—アヒル由来非定型ロタウイルス（F-29株）を用いて、鶏ひなへの感染性、及び全国各地から1988-89年に収集された鶏血清1,040例中の抗体保有状況を、間接蛻光抗体法で調べた。F-29株を経口投与された鶏ひな及び同居ひなには抗体が認められ、感染が成立した。野外鶏血清の抗体陽性率は59.3%を示した。抗体陽性率は20-40日齢でほぼピークに達し、地域間、鶏種間で明らかな差を認めなかった。種鶏群由来血清の陽性率は58.5%であった。