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A Survey of Chicken Sera for Antibody to Fowl Adenovirus Serotypes 1 and 8, Isolated from Gizzard Erosion

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Summary

Distribution of fowl adenovirus (FAV) serotypes 1 and 8, which were both isolated from gizzard lesion of broilers, was examined by serological tests, such as neutralization (NT) and agar gel precipitation (AGP) tests, using 1,159 sera collected from all over the country. In neutralization test, positive rates of serotype 1 and 8 were 35.5% and 21.3%, respectively, however both were detected without any relations to the sampling place. Antibody positive rates were higher in AGP test than in NT test, and increased according to chicken ages.

Key words: fowl adenovirus, gizzard erosion, serological survey

Introduction

Adenoviruses consist of four genera, *Aviadenovirus, Mastadenovirus, Atadenovirus* and *Siadenovirus* [2]. Fowl adenovirus (FAV) belongs to *Aviadenovirus* and has been well known as an agent of inclusion body hepatitis (IBH) in chicken, and in which 12 serotypes have been recognized in the world [9]. Serotypes of FAVs isolated from IBH are not restricted to a certain serotype but belong to many serotypes [9]. Recently, epizootic outbreaks of gizzard erosion in broiler flocks caused by fowl adenovirus infection have been reported in Japan [1, 7, 12]. The first adenoviral gizzard erosion was reported in layer chickens in 1993 [11]. Most of the FAV isolates from the gizzard lesions were serotype 1, but a few were serotype 8 [6, 12]. The gizzard lesions were reproduced in broiler and specific pathogen-free (SPF) chickens by oral infection with serotype 1 easily, but not with serotype 8 [12]. However, some young chicks orally inoculated with serotype 8 could produce mild gizzard erosions [3]. From these reports, the serotype 1 has been considered to play a main role in the occurrence of gizzard erosion.

Distribution rate of each serotype in Japan was shown in 1965, by Kawamura et al. [8]. They showed serotype 1 (prototype strain Ote) occupied 38.8% of all isolated FAVs, and followed serotype 8 (prototype strain TR59) with 20.7%. Since then, there has been no data on the distribu-

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tion of serotypes of FAV in Japan.

In this study, the distribution of FAV serotypes 1 and 8 was examined by serological tests, such as neutralization (NT) and agar gel precipitation (AGP) tests, using 1,159 sera collected from all over the country.

Materials and Methods

Chicken sera

Sera were collected from broiler and layer chickens aged 30 days or more, which were raised in 120 farms in 23 prefectures, from November, 2003 to January, 2004. Six or more sera were tested per flock. These sera were treated at 56℃ for 30 min before use, and were diluted 1:40 in physiological buffered saline for neutralization test.

Viruses

Two strains of FAV, strain JM1/1 of serotype 1 and strain B13-1 of serotype 8, which were both isolated from gizzard lesion of broilers [12], were used in the present experiments.

Serological tests

Serological tests were employed by AGP and NT tests. AGP was done by using 4 units of antigen prepared by chorioallantoic membranes (CAM) infected with strain JM1/1 as reported previously [10]. The reaction was performed in a moisture box at 37℃ overnight and then at room temperature for 2 days, and precipitin lines were observed daily with reverse lighting. AGP test is lower in sensitivity than NT test, but is specific and useful to detect antibody to any serotype.

NT test was done by mixing 40-fold diluted serum with 200 $TCID_{50}$ of each virus solution and inoculation on 48-well microplate containing chicken kidney cultured cells in ordinary methods. Two wells were used for one sample and CPE was observed on 5th day post-inoculation. The sample was judged as antibody-positive when CPE was not observed in both wells.

Results and Discussion

The results are shown in Tables 1, 2 and Fig. 1.

As shown in Table 1, AGP antibody was found in all districts with positive rates ranging from 34.5 to 51.7%. NT antibody positive rates of serotype 1 were 17.6 - 41.1%, and those of serotype 8 were 14.5 - 36.2%. These results show that both serotypes 1 and 8 distributed all over the country. AGP antibody positive rate of total sera was 42.8%, which was higher than NT antibody positive rates of serotype 1 (35.5%) or 8 (21.3%). This result suggests that chickens might have been infected with other serotypes than serotypes 1 and 8.

Table 2 shows the positive rates in sera grouped by chicken use. AGP antibody rates were higher in layer and breeder than broiler. This might result from longer exposure period of layer or breeder in their life to FAV infection. In broiler, NT antibody positive rate of serotype 1 (25.2%) was higher than that of serotype 8 (6.5%). This result might be reflected on the current onsets of gizzard erosion of broiler.

Fig. 1 shows the results at different ages in layer chickens. Data from broiler was not shown here, since broiler's life is usually very short as less than 55 days of age in Japan. Positive rates increased according to ages in both AGP and NT of serotype 1.

Twelve distinct serotypes have been recognized in fowl adenovirus. According to the report of Kawamura et al. [8], a significant cross reaction was only recognized between serotypes 2 and 3, additionally a weak cross reaction was observed between serotypes 6 and 7, 6 and 8, 2 and 5, or 2 and

Table 1. Prevalence of antibody to FAV in chicken sera from different districts

Antibody	District where sera were collected					Total
	Hokkaidō Tōhoku	Kantō Kōshinetsu	Tōkai Kinki	Chūgoku Shikoku	Kyūshū Okinawa	
AGP	65/173[a)] (37.6)	62/120 (51.7)	20/58 (34.5)	64/182 (35.2)	222/626 (35.3)	469/1,159 (42.8)
NT (Serotype 1)	58/173 (33.5)	48/120 (40.0)	19/58 (32.8)	32/182 (17.6)	237/576 (41.1)	394/1,109 (35.5)
NT (Serotype 8)	25/173 (14.5)	33/120 (27.5)	21/58 (36.2)	37/182 (20.3)	27/138 (19.6)	143/671 (21.3)

a) No. of sera with antibody/ No. of sera tested (%)

Table 2. Prevalence of antibody to FAV in broiler and layer chickens

Antibody	Chickens			
	Broiler	Layer	Breeder (Broiler)	Total
AGP	198/592[a)] (33.4)	205/392 (52.3)	93/175 (53.1)	496/1,159 (42.8)
NT (Serotype 1)	137/542 (25.2)	153/392 (39.0)	104/175 (59.4)	394/1,109 (35.5)
NT (Serotype 8)	14/214 (6.5)	109/392 (27.8)	20/65 (30.8)	143/671 (21.3)

a) See Table 1.

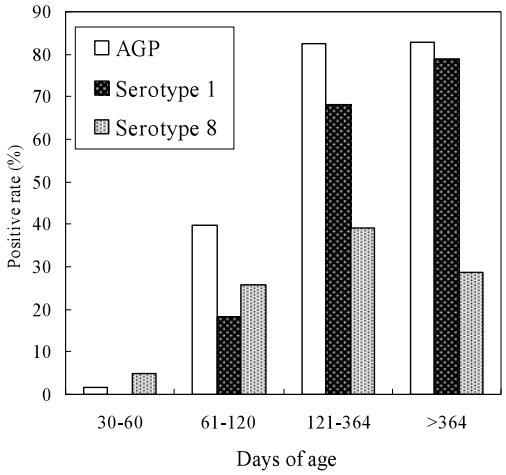

Figure 1. Prevalence of antibody to FAV in layer chicken sera at different ages.

7. Therefore, the antibodies detected in 1:40 diluted serum will be considered to be specific to each serotype.

From the results, it was shown that FAV serotypes 1 and 8 have been distributed countrywide in Japan. However, it is not clear how many cases of antibody-positive samples were related with the incidents of gizzard erosion, since not all of viruses of each serotype can produce the lesions.

These results also suggest that it might be difficult to eradicate both serotypes of FAV from broiler flocks and to prevent the occurrence of adenoviral gizzard erosion. Ono et al. [4, 5] reported that maternal antibody could not protect FAV infection, but live virus infection could protect re-infection experimentally. Therefore, a live vaccine will be preferable to a killed vaccine for protection against adenoviral gizzard erosion.

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