

A Serological Survey of Turkey Rhinotracheitis Virus Infection in Chickens in Japan

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(Received 15 December 1995/Accepted 1 March 1996)

ABSTRACT. A total of 4,111 chicken sera collected from 1985 to 1995 at 137 farms in 36 prefectures were subjected to an enzyme-linked immunosorbent assay (ELISA) to test for antibodies against turkey rhinotracheitis (TRT) virus. The antibodies to TRT virus were detected in the chicken sera collected from 1988 to 1995, but the antibodies to TRT virus were undetectable in the sera of chickens collected before 1987. The antibody positive rates each year ranged from 2.2% (1988) to 54.2% (1995). Recently, the TRT virus has spread over the flocks of commercial broiler and layer chicken throughout the country. The serological findings indicated that the TRT virus had invaded the chickens before 1988, and has been widespread thereafter in Japan. — **KEY WORDS:** ELISA, serological survey, TRT virus.

J. Vet. Med. Sci. 58(7): 689–691, 1996

Turkey rhinotracheitis (TRT) virus is considered to be a factor in swollen head syndrome (SHS) in chickens [4, 8]. In Japan, the SHS was first recognized in Hyogo prefecture in 1989 [7]. It was not long before an associated agent was isolated from commercial broiler chicken in Miyazaki prefecture in 1994 [6].

A neutralization test and enzyme-linked immunosorbent assay (ELISA) are commonly used for the serological diagnosis of TRT virus infection in chickens. ELISA has some advantage over the conventional methods, as many sera from field samples can be treated much more easily than in the neutralization test and time is saved. It is reliable and useful for detecting antibodies in the field serological survey.

In the present study, we carried out a serological survey of TRT virus infection using chicken sera collected from 1985 to 1995 by ELISA for detecting TRT virus antibodies, and to estimate the time when the TRT virus infection become prevalent among chickens in Japan.

ELISA was carried out by the modified methods of Tanaka *et al.* [5]. ELISA antigen was a purified virus of 8597/CV94 strain [6] solubilized by 0.1% sodium deoxycholate. The solubilized ELISA antigen for antibody detection appeared specific for TRT, since no detectable antibodies in the antisera against several pathogens other than TRT virus were demonstrated. To determine cut-off values, 50 chicken serum samples from various sources, which were negative for TRT virus antibody by neutralization test, were tested with the result of a mean value of 0.09 ± 0.03 . A cut-off value of 0.15 (mean + 2SD) was adopted for evaluating subsequent ELISA results. Thus, ELISA values of >0.15 were regarded as positive. The ELISA values of 100 individual chicken sera were correlated with their neutralizing antibody titer with a correlation coefficient of 0.62. The rates of coincidence and discrepancy between neutralization test and ELISA are shown in Table 1. The antibody titer higher than 1:2 was taken as positive for neutralization test. The results obtained by the neutralization test coincided with those of ELISA in the 96 of 100 samples. The rest of the 4 samples were positive for only neutralization test.

A total of 4,111 sera were randomly collected between 1985 and 1995 from broiler and layer chickens of 0-day to 500-day-old which had been no history of clinical evidence on TRT virus infection at 137 farms in 36 prefectures. The sera were submitted for serological survey to examine antibody response after various vaccinations and to test antibodies against several pathogens. They were stocked at our laboratory.

Table 2 summarizes the results of the serological survey on TRT virus infection in chickens. The antibodies to TRT virus were undetectable in the chicken sera collected before 1987. The first presence of antibodies to TRT virus was confirmed in the sera of chickens collected from Tokushima prefecture in 1988. At the time of first occurrence of SHS in 1989, chickens positive for TRT antibody were detected in Ibaraki, Hyogo, Ehime, Tokushima and Kumamoto prefectures. The antibody positive rates in chickens each year ranged from 2.2% in 1988 to 54.2% in 1995. The antibody-positive rate tended to increase from year to year. The antibody-positive rate in farms before 1991 were considerably lower than those between 1993 and 1995. The TRT virus antibody-positive chickens were considered to be distributed in most of the prefectures (administrative divisions) of Japan from 1988 to 1995. TRT virus infections was prevalent in the country among commercial broiler and layer chicken flocks.

After 1989 in Japan, outbreak of SHS in broiler chickens was reported in Yamaguchi prefecture in 1990 [1], and then

Table 1. Comparison of results between neutralization test and ELISA

Results of test		No. of serum samples (%)	
Neutralization test	ELISA		
Positive	Positive	74	} (96%)
Negative	Negative	22	
Positive	Negative	4	} (4%)
Negative	Positive	0	
Total		100	

Table 2. Annual distribution of TRT virus antibody in chickens from 1985 to 1995 in Japan

District	Number of farms	Number of sera	Year										
			1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995
Hokkaido	4	60	. ^{a)}	2/10 ^{b)}	2/10	.	.	0/30	0/10
Aomori	7	170	.	.	.	0/20	0/20	0/20	0/20	.	0/10	10/40	38/40
Akita	2	60	0/10	.	.	0/50	.
Iwate	3	130	0/60	.	.	0/30	36/40
Miyagi	1	40	0/40
Yamagata	1	10	0/10
Fukushima	1	11	0/11
Niigata	2	90	.	.	.	0/50	0/40
Gunma	1	40	0/40
Tochigi	1	10	0/10
Ibaraki	6	180	.	.	.	0/20	7/50	9/20	4/10	.	18/40	0/40	.
Chiba	6	140	0/10	0/40	0/10	.	.	35/40	40/40
Tokyo	2	30	.	.	.	0/10	0/20
Saitama	3	100	0/20	0/40	0/40
Yamanashi	2	30	2/20	0/10
Shizuoka	3	71	0/41	0/10	3/20
Gifu	6	170	.	.	.	0/80	0/20	0/10	0/10	.	0/30	.	0/20
Aichi	7	100	.	.	.	0/10	0/20	7/30	0/10	.	0/10	.	0/20
Mie	5	110	.	.	.	0/10	0/10	0/40	0/50
Fukui	1	50	16/50	.
Kyoto	2	50	.	.	.	0/10	23/40	.
Nara	3	90	.	.	.	0/20	0/40	3/30
Wakayama	1	10	0/10
Hyogo	10	280	0/20	0/10	0/40	0/40	5/50	13/40	0/10	.	8/10	40/40	20/20
Okayama	1	40	40/40
Yamaguchi	1	30	0/30
Kagawa	5	190	.	.	.	0/40	.	.	0/10	.	0/50	0/50	25/40
Ehime	5	90	.	0/20	0/20	.	5/20	.	0/20	.	0/10	.	.
Tokushima	8	260	0/40	0/20	0/40	10/40	8/60	.	0/20	.	6/10	.	0/30
Fukuoka	3	120	.	.	0/60	.	.	0/30	28/30
Saga	4	154	0/10	.	0/74	.	.	0/60	0/10
Nagasaki	2	30	0/20	0/10
Ohita	3	90	.	.	.	0/10	0/40	39/40
Kumamoto	7	365	0/75	0/50	.	0/40	10/60	0/40	0/20	.	.	50/80	.
Miyazaki	10	370	0/40	0/50	0/60	0/20	0/40	6/50	9/10	.	10/10	45/50	32/40
Kagoshima	8	340	0/40	0/50	0/60	0/40	0/20	0/40	.	.	25/50	32/40	.
Postive rate in chickens (%)			0/215 (0.0%)	0/200 (0.0%)	0/280 (0.0%)	10/460 (2.2%)	35/542 (6.5%)	40/510 (7.8%)	18/384 (4.7%)	.	67/250 (26.8%)	251/720 (34.8%)	298/550 (54.2%)
Positive rate in farms (%)			0/5 (0.0%)	0/6 (0.0%)	0/6 (0.0%)	1/16 (6.3%)	5/21 (23.8%)	6/20 (30.0%)	4/18 (22.2%)	.	5/11 (45.5%)	8/16 (50.0%)	9/18 (50.0%)

a) Not tested.

b) Number of ELISA positive sera/Number of sera tested.

in Hyogo prefecture in 1993 and 1994 [2]. Ogura *et al.* [2] confirmed the antibody to TRT virus in SHS-affected chickens by ELISA.

Otherwise, Ootsuki reported an antibody survey of TRT virus infection using chicken sera collected between 1989 and 1994 by a neutralization test. He indicated that the virus had already spread among chickens throughout Japan [3]. In this survey the antibody positive rates in chickens were similar to those in our works. ELISA has been proved to be satisfactory for use in serological surveys of TRT virus infection in the field chicken flocks.

The findings of this serological survey suggest that the invasion of TRT virus among the chickens had occurred

before 1988. Presumably that the TRT virus infection had already occurred among chickens in 1988, and has been widespread in Japan thereafter. Knowledge of the pathogenicity and infectious route of TRT virus in fields is meager at present. Further precise studies are required on the role of the virus in the SHS outbreak and their control.

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