Chlamydial Antibodies in Domestic Birds Detected by Indirect Hemagglutination Tests

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Chlamydial Antibodies in Domestic Birds Detected by Indirect Hemagglutination Tests

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SUMMARY

An indirect hemagglutination test for detecting avian chlamydial antibodies was established. Sensitized red blood cells were prepared using sodium N - laurylsarcosinate soluble fraction of purified elementary bodies and glutaraldehyde prefixed sheep red blood cells by glutaraldehyde method. The established indirect hemagglutination test revealed chlamydial antibodies in various poultry. Positive rates were 17.0% (423/2491) in chickens, 48.5% (32/68) in ducks, 50.0% (3/6) in turkeys, 25.0% (130/520) in quail and 15.8% (9/57) in pheasants, respectively. It is suggested that chlamydial infections of domestic birds may be widespread in Japan, and that the birds may be serve as potential revervoir hosts for human zoonotic chlamydial infections.

INTRODUCTION

Chlamydia psittaci is known to infect a variety of birds, animals, and humans. One hundred and thirty species of birds are considered to be avian hosts of *C. psittaci*[1]. Psittacine birds including parrots, mascows, and budgerigars are considered as the most important reservoir hosts for human chlamydiosis, psittacosis[2]. Other domestic birds such as ducks, turkeys and meat-type pigeons are also reported as the sources of psittacosis in European countries and the United States[3].

Survey of infectious diseases in imported and domestic birds and animals by our laboratory has revealed the widespread chlamydial infections in pet birds[4~7], feral pigeons[8], cats and dogs[9] and domestic animals[10]. However, chlamydial infections in domestic fowls, in spite of the importance of these birds as meat birds in public health, have not been reported in Japan as yet.

It is generally said that avian immunoglobulins do not fix guinea pig complement[11]. Therefore, the complement fixation (CF) test, which is the most popular method for detecting chlamydial antibodies, is difficult to apply for survey of chlamydial antibodies in domestic fowl. Indirect hemagglutination (IHA) test is a simple method requiring only sensitized red blood cells (RBC) for examination, and is applicable to serological investigations in birds and animals whether immunoglobulins fix guinea pig complement or not[12]. Some authors have reported the usefulness of IHA test to detect chlamydial antibodies in animals[13~17]. However, IHA test has not been applied to detect chlamydial antibodies in birds. Therefore, establishment of the IHA test for detection of antibodies to *Chlamydia psittaci* and survey of chlamydial antibodies in domestic fowls using IHA are described in this report.

MATERIALS AND METHODS

Antigens : Elementary bodies (EB) of *C. psittaci* GCP-1 strain were grown and purified as described elsewhere[8]. Sensitizing antigens were prepared from detergent solubilized lysates of the

purified EB as follows : The purified EB at protein concentration of 2 mg/ml was mixed with an equal volume of 2% (w/v) detergent to incubate at 37℃ for 30 min. The solubilized EB lysates were divided into soluble and insoluble fractions by centrifugation at 12,000 g, for 30 min. Detergents for solubilization were sodium N-laurylsarcosyinate (Sarcosyl), sodium dodecylsulfate (SDS), sodium deoxycholate, Triton X-100, Tween 20 and Tween 80.

Sensitized RBC : Sensitized RBC were prepared by the standard methods[18]. One volume of sheep RBC washed 3 times with PBS was suspended in 10 volumes of PBS to which 1 volume of 2.5% glutaraldehyde solution was gradually added with stirring. After 30 min with stirring, the RBC were washed 5 times with PBS and stored as 10% prefixed RBC suspension in PBS containing 0.1% sodium azide at 4℃ until use. One volume of 10% prefixed RBC suspension was centrifuged to obtain RBC as a pellet which was mixed with one volume of antigen solution. Then 1.5 volume of 1% glutaraldehyde solution was added dropwise with stirring for 60 min at room temperature. After two washings with PBS, the RBC were incubated in 0.01M Tris-HCl buffered saline, pH 7.2 for 30 min. Washing twice with PBS, the RBC were finally suspended in PBS as 1% (v/v) suspension of sensitized RBC.

IHA test : IHA test was done by the microtiter method as 0.1 ml reaction volume. Titers of 1 : 16 or higher were considered as a positive reaction.

Sera examined : Sera of five avian species were tested (Tables 2 and 4). Chicken collected from 1956 to 1984 were kindly provided by the National Institute of Animal Health, Aichi Prefectural Institute of Public Health, Omiya Live Stock Hygiene Service Center, Nippon Institute for Biological Science, Agri-Industry Laboratory and Chemo-Sero-Therapeutic Research Institute, respectively. Duck sera were kindly provided from National Institute of Animal Health and Omiya Live Stock Hygiene Service center. Quail sera were a gift of Research Laboratory Chikusan-Kono-Sha Co., Aichi. Pheasant and turkey sera were contributed by the National Institute of Animal Health. SPF chicken sera were obtained from the Nippon Institute for Biological Science, and Aburahi Laboratory of Shionogi Co., Ltd. All sera examined were inactivated at 56℃ for 30 min.

Experimentally immunized chicken sera : Two chickens were intramuscularly immunized with purified EB (100 μg of protein) emulsified with Freund's complete adjuvant. Other chickens were injected with homogenates of normal yolk sac for control group. Sera were bled at 3-day intervals from 6 to 24 days after injection.

Enzyme linked immunosorbent assay (ELISA) for avian sera : ELISA was performed as described elsewhere[19] with minor modifications : Anti-chicken IgG goat antiserum was used as secondary antibody to detect chicken IgG.

RESULTS

Establishment of IHA tests : The prefixed RBC were sensitized with Sarcosyl soluble supernatants of EB. The glutaraldehyde and tannic acid methods were used for glutaraldehyde and formaldehyde prefixed RBC, respectively. Although formaldehyde-prefixed tannic acid sensitized RBC showed non-specific reactivities, 2.5% glutaraldehyde-prefixed 1.0% glutaraldehyde sensitized RBC showed specific IHA reactivity. The sheep RBC were sensitized with whole lysates, soluble supernatant fluids and insoluble pellets of solubilized EB solutions. A preliminary examination indicated that soluble supernatant fluids of SDS, Sarcosyl, and Triton X-100 lysates would have high antigenic reactivity in IHA assay. In quantitative experiments, the Sarcosyl soluble supernatant fluid at a protein concentration of 12.5 μg/ml gave the highest specific IHA activity for immunized chicken serum (Table 1). Therefore, the sensitized sheep RBC were prepared by glutaraldehyde method with glutaraldehyde prefixed RBC and supernatant fluid of Sacrosyl solubilized purified EB at a protein

concentration of 12μg/ml.

Table 1. Hemagglutination titers of rabbit hyperimmune serum with RBC sensitized by detergent soluble solutions of purified EB of *C. psittaci* GCP-1 strain.

Detergent	Protein concentration (μg/ml)	Agglutination titers*	
		Immune serum	Normal serum
SDS	78	512	512
	40	512	512
	20	128	64
	10	32	16
	5	32	<8
	2.5	32	<8
Sarcosyl	50	256	64
	25	128	8
	12.5	64	<8
	6.3	32	<8
	3.2	32	<8
	1.6	32	<8
Triton X-100	60	256	32
	30	64	8
	15	32	<8
	7.5	32	<8
	3.8	32	<8
	1.8	32	<8

* Agglutination titers were expressed as the reciprocal of the highest dilution of sera that agglutinated sensitized RBC.

Comparison of IHA and other immunoassays : A correlation of IHA assay and ELISA was investigated by tracing antibody response of immunized chicken (Fig. 1). IHA antibodies were detected from 6 days post infection and reached the highest titer of 1 : 256 at 12 days post infection. The ELISA antibody ascent paralleled that of IHA antibody at first, but it was maintained for a

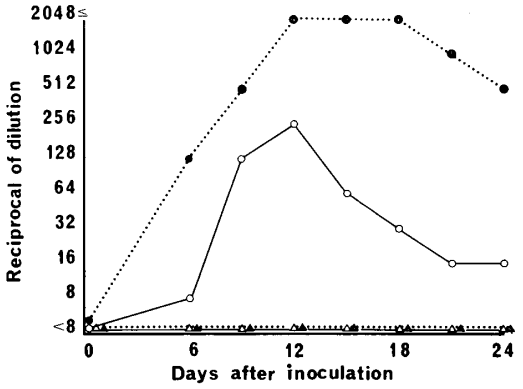

Fig. 1. Antibody responses of immunized chickens. Chickens aged 2-weeks were immunized with purified EB of *C. psittaci* GCP-1 strain. Serum samples were taken at 3-day intervals from after 6 days of inoculation. Serum antibodies of immunized (○,●) and control (△,▲) chickens were titrated by IHA tests (○,△) and ELISA (●,▲).

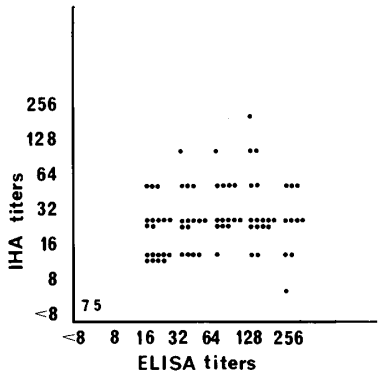

Fig. 2. Comparison of chicken antibody titers of IHA test and ELISA. One hundred and fifty sera were titrated by both methods and plotted.

Table 2. Chlamydial IHA antibodies in chicken from 1956 to 1984

Years	Prevalence of positive sera	Agglutinating titers					
		≦1 : 8	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128≦
1956-64	6/ 63(9.5)	52	5	5	1	0	0
1965-71	6/ 146(4.1)	122	18	6	0	0	0
1972	82/1231(6.7)	1056	93	53	22	7	0
1981	88/ 389(22.6)	219	82	60	16	9	3
1983	53/ 366(14.5)	206	102	34	15	3	1
1984	171/ 296(57.8)	59	54	79	56	15	21

longer period. IHA and ELISA antibody titers of randomly collected chicken sera showed a high correlation as seen in Fig 2. Out of 75 ELISA positive sera, 74 were positive in IHA tests, and ELISA negative sera were all negative in IHA tests. A correlation coefficient of chicken sera was 0.87. These results indicated that IHA assay could be applicable to detect chlamydial antibodies of chicken sera.

Epidemiological survey of domestic fowls : Seroepidemiological survey was made of various domestic fowl using IHA tests. Positive rates of chickens varied from 4.1 to 57.8% (Table 2). Annual distribution of the positive rates ranged from 14.5 to 57.8% in recent years, against 4.1 to 9.5% before 1972. The breeding flocks showed higher antibody positive rates than those of white Leghorns and broilers (Table 3). Regional differences were observed in the positive rates of chickens. Higher positive rates were observed in Chubu (41.6%) and Hokkaido (28.6%) districts in Japan. On the other hand, lower positive rates were obtained in Kanto (18.9%) and Shikoku districts (14.0%).

Table 3. Distributions of chlamydial IHA antibodies in chickens

Chickens	year	Prevalences of positive sera
White Leghorn	1981	88/389(22.6)
	1984	8/ 39/20.5)
	total	96/428(22.4)
Broiler	1983	35/255(13.7)
	1984	6/ 39(15.4)
	total	41/294(13.9)
Breeding flocks	1984	170/218(78.0)

IHA antibodies in other domestic fowl are shown in Table 4. Positive rates of ducks were 48.5%. Duck sera from Saitama Prefecture

Table 4. Distribution of chlamydial IHA antibodies in domestic fowl

Fowls	Places	Prevalence of positive sera	Agglutinating titers						
			≦1 : 8	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256≦
Ducks*	Saitama	11/ 15 (80.0)	2	1	5	5	1	0	1
	Kyoto	13/ 19 (68.4)	0	6	12	1	0	0	0
	Osaka	8/ 34 (23.5)	7	19	5	2	1	0	0
	Total	32/ 68 (48.5)	9	26	22	8	2	0	1
Turkeys†	Fukuoka	3/ 3 (100)	0	0	2	1	0	0	0
	Kochi	0/ 3 (0)	3	0	0	0	0	0	0
	Total	3/ 6 (50.0)	3	0	2	1	0	0	0
Quails‡	Okazaki	130/520 (25.0)	179	211	97	27	6	0	0
Pheasants§	Okayama	2/ 5 (40.0)	2	1	0	1	1	0	0
	Gifu	5/ 24 (20.8)	18	1	4	1	0	0	0
	Hiroshima	2/ 28 (7.1)	26	0	0	2	0	0	0
	Total	9/ 57 (15.8)	46	2	4	4	1	0	0

Sera were collected in 1979 and 1984 for ducks (*), 1980 and 1984 for turkeys (†), 1984 for quails (‡), and 1980 for pheasants (§).

showed higher positive rates than those from other places. Positive rates of turkeys and quail were 50.0% and 25.0%, respectively. Pheasants had chlamydial antibodies at a rate of 15.8%. Pheasant sera from Okayama Prefecture showed higher positive rates than those from Gifu and Hiroshima Prefectures.

DISCUSSION

Chlamydial antibodies in mammalian species including humans have been detected by CF test. The standard method of CF test is not able to detect avian immunoglobulins which do not fix C'1 component of guinea pig complement so that modifications of CF test or hemagglutination inhibition (HI) tests which use murine RBC have been used to detect chlamydial antibodies in avian species. The modifications for CF test develop direct CF[20], indirect CF[21] and supplementary CF[22] tests which require great skill to obtain reproducible results. It is difficult to obtain murine RBC for HI tests constantly.

IHA test is a feasible method to detect chlamydial antibodies in birds. The sensitizing antigen in the present study is shown to contain genus specific antigens (Fukushi et al. in preparation). Therefore, the established IHA could detect chlamydial antibodies in avian species. The antibody titers obtained by IHA test and ELISA were correlative. Since IHA test must use RBC, RBC types could be a cause of nonspecific reactivity. This nonspecific reaction could be decreased by using RBC of adequate animals and other ligands such as Latex particles. The IHA test described here would be a valuable aid to detect specific chlamydial antibodies in avian species.

In the present survey, high degrees of antibody possession were revealed in domestic fowl by survey for the first time in Japan. Chickens, which are important species in public health as food and egg sources, have possessed chlamydial antibodies in high percentages. Furthermore, the positive rate becomes higher in accordance with the advance in year. Although psittacosis caused by chickens have rarely been reported[3,23,24], chickens might be considered as potential sources for human chlamydiosis because of a large number of reproductions.

There were 95,000 domestic ducks in 1976 in Japan[25]. Most of them are special products in Osaka and Saitama Prefecture. The antibody possession rate obtained in this examination (48.5%) was as high as a result (63%) of Evans *et al.* using ELISA[26]. Ducks are waterfowl among domestic birds, so the possibility of water-born transmission must be considered. Chlamydia-like particles have been observed in several aquatic animals[27,28]. Comprehensive epidemiological survey of domestic animals, domestic fowl, waterfowl, reptiles and aquatic animals may be required to resolve the transmission cycles and ecology of *C. psittaci* in nature.

No chlamydial infection of quail has been reported so far, in spite of high antibody possessing rates as described here. Because 70% of total quail production in Japan derives from Aichi Prefecture in Chubu district[25] where higher antibody possessing rates in chicken were observed, further survey is needed to reveal the mechanism of chlamydial infections in quail.

Turkeys are known to be an important source of zoonotic human chlamydiosis, especially in poultry workers in the United States[3]. Only 4,807 turkeys were fed in 1976 in Japan[25]. A small number of turkeys examined showed 50% antibody positive rates in the present study. Pheasants numbered 154,500 in Japan in 1976[25] had fewer chlamydial antibodies in this survey. Page[1] described a human chlamydial infection caused by pheasants. Therefore, continuous observation of this avian species is needed as potential reservoir hosts of zoonotic chlamydial infections.

Reports on the chlamydial infections of humans and animals are fewer in Japan than in other countries. It is interest to note the widespread growth in the number of chlamydial antibody

possessing birds and animals in Japan (e. g., present and previous research)[4~10]. The IHA test described here requires no special skill and can be done quickly. It would be a valuable tool to investigate chlamydial infections in various birds and animals including humans.

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間接赤血球凝集反応による鳥類のクラミジア抗体の検出

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(1985年7月31日受理)

要　　約

鳥類のクラミジア抗体を検出するため間接赤血球凝集反応の確立を試みた。感作赤血球は N-ラウリルザルコシン酸ナトリウム可溶性基本小体抗原をグルタールアルデヒドで前固定した羊赤血球に感作して作製した。この感作赤血球を用いた凝集反応により，ニワトリ，アヒル，シチメンチョウ，ウズラおよびキジからそれぞれ17.0% (423/2491)，48.5% (32/68)，50.0% (3/6) および25.0% (130/520) の率で抗体が検出された。これらの鳥類もヒトのクラミジア感染症の感染源になる可能性について考察した。

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