Survey of Infectious Coryza of Chickens in Indonesia

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ABSTRACT. A survey of infectious coryza of chickens was performed in West Jawa of Indonesia between 1987 to 1988 by the detection of hemagglutination-inhibition (HI) antibody against *Haemophilus paragallinarum* (Hpg) in non-vaccinated healthy laying (12 farms, 196 chickens) and native (8 farms, 197 chickens) chickens. HI antibodies against Hpg were detected not only in the native chickens but also in the laying chickens, regardless of the district, and were observed in 70% (14/20) of farms and 19% (73/393) of chickens. HI antibodies against serotype A were detected from 11 farms (55%) and 11% (45/393) in chickens. Those against serotype C were detected from 5 farms (25%) and 8% (30/393) in chickens. Three Hpg strains were isolated from different chickens affected with infectious coryza. Two of them were identified as type A and the other as type C by the rapid plate agglutination test. These results demonstrated that the outbreaks of infectious coryza caused by serotype A and C strains had occurred in Indonesia.—**KEY WORDS:** *Haemophilus paragallinarum*, HI antibody, infectious coryza.

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Infectious coryza (IC) was an acute respiratory disease of chickens caused by *Haemophilus paragallinarum* (Hpg), and clinically characterized by nasal discharge and facial swelling. IC may occur in growing and laying chickens. The greatest economic losses result from increased number of culls and marked reduction in egg production. Hpg was recognized as a cause of IC by Eliot and Lewis [2] in 1934. Since then, many workers isolated Hpg and demonstrated the wide spread of IC in the world [1, 3, 7, 11, 13]. Effective inactivated vaccines prepared from Hpg strains of serotypes A and C have been widely used for the prevention of IC in Japan [9, 10].

In Indonesia, prevalence of IC has become a major problem in chicken production and the commercial inactivated vaccines prepared from Hpg strains of serotypes A and C are also used for prevention of the disease. However, there is no information about survey of IC of chickens and the epidemiological features of the disease are still unclear in Indonesia.

This report deals with the survey of hemagglutination-inhibition (HI) antibody in chickens and characterization of Hpg strains isolated in Indonesia between 1987 to 1988.

MATERIALS AND METHODS

Source of chicken and sera: In 1987 and 1988, we collected a total of 393 serum samples from native

and laying chickens on 20 farms in 4 different geographical locations: Bogor, Cianjur, Bukasi and Jakarta of West Jawa. Those chickens were not immunized with IC vaccine. The condition of their flocks is shown in Table 1. The native chickens were kept in pasture on the farmhouses and their ages were unidentified. The laying chickens were kept in the cages. None of the chickens in all farms showed clinical signs of coryza at the survey. For the bacterial isolation, we selected 3 other farms shown in Table 3 in Bogor, because an outbreak of IC has continued in these farms. These chickens were not immunized with IC vaccine. The bacterial isolation was achieved about 3–5 days after the appearance of the signs.

Bacterial isolation: Nasal swabs were collected from 10 chickens affected with infectious coryza and streaked onto chicken-meat infusion (CMI) agar (chicken meat infusion, 1.0% polypeptone-S, 0.5% NaCl, 1.5% agar, pH 7.2) plate containing 5% sterilized chicken serum. The plates were incubated in 10% CO₂ atmosphere at 37°C for 18 hr. Hpg-like colonies were selected and subcultured. Gram-stain reaction was done for the observation of cell morphology. Strains 221 and Modesto of Hpg were used as reference [15]. The latter strain was kindly provided by Dr. K. Kume of The Kitasato Institute, Japan. All the isolates were propagated in 5-day-old embryonated egg. Infected yolk was harvested at death of embryo, and stored at –80°C until use.

Agglutination test: For serotyping, rapid plate agglutination (RPA) test was employed [16]. Agglutinogens of each isolate and reference strain were prepared as follows: the bacteria were grown in 20% CMI broth (20% chicken meat infusion, 1% polypeptone-S, 0.5% NaCl, pH 7.2) containing 1% chicken serum and 1% yeast extract in 10% CO₂ atmosphere at 37°C for 24 hr. The bacterial cells were collected by centrifugation of 10,000 × g for 20 min and suspended in phosphate-buffered saline (PBS, pH 7.2) containing 0.01% thimerosal. The cell suspension adjusted to the concentration of 10 times the optical density of 0.4 at 540 nm was designated as an agglutinin. For the preparation of antiserum against strains 221 and Modesto, 35-day-old specific-pathogen free (SPF) chickens (Nippon Institute for Biological Science, Line-M) were immunized 3 times with killed bacteria (10⁸ CFU/ml) adjuvanted with aluminum hydroxide gel, at 2 weeks intervals.

Preparation of hemagglutinin: Hyaluronidase-treated hemagglutinating (HA) antigens for HI test were prepared by the method of Yamaguchi *et al.* [15] using the isolates and the reference strains.

HI test: To determine HI antibody, HI test was performed as described by Yamaguchi *et al.* [15] using hyaluronidase-treated HA antigens prepared from the reference strains and formaldehyde-fixed chicken erythrocytes. Titer in HI test was expressed as the reciprocal of the highest dilution showing complete inhibition of HA. The HI titer of 1:5 or higher was considered positive.

Pathogenicity test: Seven-day-old SPF embryonated egg were inoculated with 0.1 ml of a broth culture (approximately 10⁸ CFU/ml) via the yolk sac. Thirty-five-day-old SPF chickens were administered with 0.1 ml of the culture (at least 10⁷ CFU/0.1 ml) via the nasal cavity. Death in the embryos or appearance of clinical signs of coryza such as nasal discharge and facial swelling in the chickens was recorded.

Antimicrobial susceptibility of the Hpg isolates: The minimal inhibitory concentration (MIC) of antimicrobial agents against the Hpg isolates was determined by the agar dilution method [4] using Brain Heart Infusion agar (Eiken, CO., Tokyo) containing 5 µg/ml nicotinamide adenine dinucleotide (NADH, Oriental Yeast Corp., Japan) and 5% chicken serum. Two-fold serial dilution of antimicrobial stock solutions was prepared so that concentrations of antimicrobial agents ranged from

0.2 to 100 µg/ml. The antimicrobial agents used were dihydrostreptomycin (DSM), kanamycin (KM), spiramycin (SP), erythromycin (EM), novobiocin (NB), chloramphenicol (CP), penicillin-G (PCG), ampicillin (ABPC), tetracycline (TC), oxytetracycline (OTC), bacitracin (BC) and colistin (CL). The isolates and reference strains were grown in 20% CMI broth containing 3% chicken serum at 37°C for 18 hr. After inoculation of the bacteria onto the agar plate, the plates were incubated in 10% CO₂ atmosphere at 37°C for 48 hr. The isolation of plasmid was performed by the method of Kado and Liu [6].

RESULTS

Positive rates of HI antibody against Hpg: Origin of sera for the detection of HI antibody against Hpg was shown in Table 1. HI antibody against Hpg was detected from the chickens in all geographic areas, but there were some farms on which the HI antibody against not only serotype A but also serotype C (Table 1) could not be detected. In the native chickens, the number of chickens positive for HI antibody against serotype C was more than that against serotype A. In the laying chickens, that against serotype A was more than that against serotype C. The positive rate of antibody on each farm in the laying chickens ranged 14–59%, and these titers were higher than those of the native chickens (8–30%). Only two sera of the laying chickens had the HI antibodies against both types A and C, and another serum had the HI antibodies against either serotype A or C.

Table 2 shows the positive rate of HI antibody against Hpg. The number of farms where the HI antibody against serotype A was detected was more than that of serotype C. The total positive rate of HI antibody against Hpg was 75% (6/8) in farms and 20% (40/197) in chickens.

Isolation of Hpg and their biological properties: In 1988, 3 Hpg strains were isolated from the chickens affected with IC on 3 farms different from the farms shown in Table 1 in Bogor (Table 3). There was no difference in the biological properties among all isolates and strain 221 used as a reference of Hpg [17], except that strain F-1 did not ferment mannose.

All the isolates were highly pathogenic to chicken embryos and chickens. Six-day-old chicken embryos were killed within 24–30 hr after challenge-

Table 1. Origin of sera for the detection of HI antibody against *H. paragallinarum*

Area	Year	Farm	Chicken	Size of flock	Age (Month)	Number of sample	Number of chickens positive for HI antibodies against	
							Serotype A	Serotype C
Jakarta	1987	a	Native	5-60	NK ^{a)}	30	0	9
		b				9	0	0
		c				25	0	2
		d				55	0	17
		e				10	0	0
		f				9	1	0
		g				11	0	0
		h				7	1	0
		i				10	1	0
		j				10	0	0
		k				10	1	0
		l				10	1	0
Sub-total						196	5	28
Bogor	1988	m		2,000	4	26	0	0
		n		2,000	14	32	9	0
		o		3,000	20	27	16	1
Bukasi	1988	p	Laying	4,000	3	14	8	0
		q		2,000	2	36	0	0
		r		20	12	10	2	0
Cianjur	1988	s		30	12	23	1	0
		t		30	30	29	4	1
Sub-total						197	40	2
Total						393	45	30

a) Not known.

Table 2. Positive rate of HI antibody against *H. paragallinarum* serotypes A and C on the Indonesian farms

Chicken	Division	Positive rate of HI antibody (%)		
		Serotype A	Serotype C	Total
Native	Farm	5/12 ^{a)} (42)	3/12 (25)	8/12 (67)
	Chicken	5/196 (3)	28/196(14)	33/196(17)
Laying	Farm	6/8 (75)	2/8 (25)	6/8 (75)
	Chicken	40/197 (20)	2/197(1)	40/197(20)
Total	Farm	11/20 (55)	5/20 (25)	14/20 (70)
	Chicken	45/393 (11)	30/393(8)	73/393(19)

a) Number of chickens positive for HI antibody/Number of samples.

exposure. Thirty-five-day-old chickens administered with the isolates showed clinically typical signs of coryza within 24-48 hr. No significant difference was observed among the isolates and strain 221 in virulences both for chicken embryos and for chickens. Organisms were recovered from the nasal cavity and paranasal sinus of the infected chickens at 1 week after challenge-exposure.

Serological properties of Hpg strains: The results of RPA test showed that strains F-1 and F-2 belonged to serotype A, and strain F-3 belonged to serotype C (Table 3). These results were confirmed by the cross-HI test (Table 4). Strains F-1 and F-2 cross-reacted with strain 221 (type A), whereas

Table 3. Origin and serotype of *H. paragallinarum*

Name of isolate	Area	Date	Chicken	Age (Month)	Size of flock	Rate of infection (%)	Serotype ^{a)}
F-1	Bogor	6. 3.88'	Laying	14	2,000	2.2	A
F-2	"	6.29.88'	"	24	2,000	0.6	A
F-3	"	7. 1.88'	"	18	1,000	4.8	C

a)Rapid plate agglutination test was employed for serotyping.

Table 4. Cross-HI test of *H. paragallinarum*

HA-antigen ^{a)}	Antiserum				
	221	F-1	F-2	F-3	Modesto
221	80	20	20	<5	<5
F-1	40	20	20	<5	<5
F-2	80	20	20	<5	<5
F-3	<5	<5	<5	40	40
Modesto	<5	<5	<5	40	40

a) Hyaluronidase-treated antigens and formaldehyde-fixed chicken erythrocytes were employed for HI test.

strain F-3 did with strain Modesto (type C).

Antimicrobial susceptibility of Hpg strains: Table 5 shows the results of antimicrobial susceptibility of all the 3 strains including the reference strains 221 and Modesto. All the strains were highly susceptible to CP. Strain F-1 was resistant to DSM, KM, SP, EM, and CL, and moderately susceptible to PCG, ABPC, TC, OTC, and BC. Strain F-2 was resistant to DSM, ABPC, and NB, and moderately susceptible to KM, SP, EM, PCG, TC, OTC, BC, and CL. Strain F-3 was resistant to OTC, ABPC, and DSM, moderately susceptible to CP and OTC, and weakly

susceptible to SP, TC, and CL. Plasmid was not detected from the 3 strains.

DISCUSSION

The present investigation showed that HI antibodies against serotype A and serotype C strains of Hpg were detected not only in the native chickens but also in the laying chickens, regardless of the district, and that they were observed from 70% of the farms. In fact, Hpg strains of both serotypes A and C were also isolated from infected chickens in the field. Moreover, HI antibodies against both serotypes were detected from 2 chickens in 2 farms, indicating a dual infection. Our results showed that the positive rate of HI antibody against serotypes A and C was different among the kinds of chickens. The positive rate of HI antibody against serotype C in a special farm was high. This difference may be depended on the susceptibility of the native chickens. It will be necessary to investigate the susceptibility of the native chickens against Hpg. In addition, our results suggested that Hpg of serotype A was prevailed more widely than serotype C in the laying chickens. In this survey, it is indicated that

Table 5. MICs of *H. paragallinarum*

Drug ^{a)}	MIC (μ g/ml, u/ml)											
	<0.2	0.2	0.39	0.78	1.56	3.12	6.25	12.5	25	50	100	<100
DSM					○●							▲△□
KM					○●□			△				▲
SP							○●△	□				▲
EM					○●□			△				▲
NB			○●▲□								△	
CP	○●▲△□ ^{b)}											
PCG							○●▲△□					
ABPC			○●				▲			△	□	
TC			○●				▲	△	□			
OTC			○●			△	▲				□	
BC							○●▲△□					
CL							○	●□	△			▲

a) DSM: dihydrostreptomycin, KM: kanamycin, SP: spiramycin, EM: erythromycin, NB: novobiocin, CP: chloramphenicol, PCG: penicillin G, ABPC: ampicillin, TC: tetracycline, OTC: oxytetracycline, BC: bacitracin, and CL: colistin.

b) ○: strain 221, ●: strain Modesto, ▲: strain F-1, △: strain F-2, and □: strain F-3.

the outbreaks of IC caused by 2 serotypes were widely prevalent in Indonesian field.

All isolates investigated showed the same characteristics as the reference strain of Hpg, except that strain F-1 did not ferment mannose. Some isolates in Japan also demonstrated the same characteristics [8, 11]. The results of RPA test and cross-HI test showed that strains F-1 and F-2 belonged to the serotype A and were antigenically same as strain 221, and that strain F-3 belonged to serotype C and was antigenically same as strain Modesto. At present, there are two types of commercially available vaccines in Indonesia: monovalent inactivated vaccine using strain 221 of serotype A and mixed inactivated vaccine with strain W of serotype A and strain Modesto of serotype C. The present results suggested that these vaccines could be suitable for the condition in the field. Therefore, it will be possible to prevent an outbreak of IC by using effectively these commercial vaccines. Most of farms indicated single infection caused by Hpg serotypes A or C. Therefore, it is necessary to select the vaccines suitable for the condition of each farm.

The test of antimicrobial susceptibility of the isolated strains revealed resistance to DSM, KM, SP and EM and low sensitivity to OTC and TC. This tendency is also observed in Japan [14] or Australia [1], where these drugs are added to feeds [12] and use for the treatment of IC outbreaks [10]. No definite conclusion can be drawn because of a small number of isolates examined in the present study. However, the fact that they generally exhibited low or moderate sensitivity, though highly sensitive to chloramphenicol, suggests the extensive use of the similar drugs to prophylactics and remedies. Therefore, it will be necessary to instruct the people in change in proper application of these drugs in the fields. No plasmids were detected in these strains examined. This result revealed that antimicrobial drug resistance is not plasmid-mediated in these isolates, and described by Blackall [1].

Native chickens account for about 65% population of the poultry in Indonesia. They range freely in small groups in farmhouse yards. In the near future, it will be necessary to vaccinate these native chickens. All of these laying farms investigated in the present study had open-system chicken houses. The space between chicken houses was narrow, and disinfection procedures were not satisfactory. In these farms, the positive rates of infection were lower than those of previous reports [3, 7]. But,

under these conditions Hpg would spread quickly once an outbreak of disease occurs. Unfortunately, no farms in our survey gave their chickens the vaccine. Our results strongly propose that it is necessary to prevent chickens in Indonesia from IC by using the vaccine. In many farms, however, there was a problem about vaccination, especially the frequency or the interval. It is also necessary to teach them more effective vaccination methods [5, 10].

This survey is the first trial in Indonesia. We could obtain the useful information of outbreaks of IC. Since this survey covered only West Jawa, further investigation in other areas should be carried out for the decision of the kind of vaccines or vaccination program.

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