Acta virol. **31** : 138—145, 1987

SEROLOGICAL RELATIONSHIP AMONG TEN STRAINS OF AVIAN INFECTIOUS BRONCHITIS VIRUS

K. OTSUKI, Y. SAKAGAMI, M. TSUBOKURA

Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori 680, Japan

Received May 27, 1986

Summary. — Ten strains of avian infectious bronchitis virus (IBV) were studied serologically by cross-neutralization test using rabbit and chicken immune sera. With the chicken sera all 10 IBV strains were antigenically related. In particular, anti-KH serum neutralized all heterologous strains except of the Ishida strain; Nerima strain was neutralized by all antisera except of anti-Ishida serum. Most cross-reactions were less or more heterologous, thus all 10 IBV strains seemed to belong to one serological type. Using rabbit sera, all strains except of Connecticut A-5968, cross-reacted with certain other strains. Most cross-reactions were partially heterologous showing one-way-relationship; heterologous relations were observed less frequently than with chicken sera.

Key words: avian infectious bronchitis virus; serology; virus neutralization test

Introduction

In 1956, Jungherr *et al.* (1956) observed the immunological difference between Massachusetts and Connecticut strains of avian infectious bronchitis virus (IBV) by virus-neutralization test. Later on it was reported that Iowa-97, Iowa-609, Gray and Holte strains were not necessarily identical in virus--neutralization tests (Hofstad, 1958; Winterfield and Hitchner, 1962; Hitchner *et al.*, 1964). Numerous so-called "serotypes" have been reported for IBV (Cumming, 1963; Hopkins, 1969, 1978; Cowen *et al.*, 1971; Winterfield *et al.*, 1971; Johnson *et al.*, 1973, 1976; Raggi and Gomez, 1975; Johnson and Marquardt, 1976), but serotyping of the IBV has not been clearly established.

In 1974 Hopkins tested 19 representative IBV strains and grouped them into eight serotypes. Cowen and Hitchner (1975) attempted the serotyping of IBV and reported that according to serologic grouping the representative 12 IBV strains were not distinct but reflected numerous intergroup relationships. Johnson and Marquardt (1975) stated that such cross-relation as observed by Hopkins (1974) was not observed among 9 strains using the chick tracheal organ culture. There has been no other report dealing with

the antigenicity of IBV isolated in Japan than those of Kawamura *et al.* (1963) and Doi *et al.* (1982). They both stated that the antigenicity of Japanese strains was related to that of the U.S. strains. The detailed serological properties of IBV strains isolated in Japan remained obscure.

We examined the 10 IBV strains including 4 Japanese ones for their resistance to various chemical and physical agents (Otsuki *et al.*, 1979*a*), their multiplication in several cell cultures (Otsuki *et al.*, 1979*b*), interferon induction and their sensitivity to interferon (Otsuki *et al.*, 1979*c*). In the present experiment, the 10 IBV strains were tested for their possible antigenic relationships.

Materials and Methods

IBV strains. The following 10 IBV strains were tested: Beaudette-42 (Be-42), Massachusetts-41 (IB-41), Connecticut A-5968 (A-5968), Connaught, Holte, Iowa-609, KH, Nerima, Ishida and Shiga (Otsuki *et al.*, 1979*a, b, c*).

Cells. Chick kidney (CK) cells (Otsuki *et al.*, 1979*a, b, c*) were mainly used.

Immune sera. Antisera were obtained from rabbits and SPF chickens. The methods of immunization were described in previous report (Otsuki *et al.*, 1979*b*).

Virus titration. Virus titration was performed as previously described (Otsuki *et al.*, 1979*a*).

Virus neutralization tests (*VNT*) were carried out in CK cells. The sera were inactivated by heating for 30 min at 56 °C and diluted twofold from 1 : 5 to 1 : 5,120. One mililiter of virus suspension containing about 200 $TCID_{50}$ per 0.1 ml was added to 1.0 ml of each serum dilution and the serum-virus mixtures were incubated for 18 hr at 4 °C. A 0.1 ml portion of each mixture was inoculated into 4 test tubes. The end points were calculated and expressed as reciprocal of the initial dilution of serum preventing the appearance of cytopathic effect in 50 % of the CK cells.

Table 1. Cross-neutralization tests among 10 IBV strains and corresponding chicken sera

Virus strain	Antisera					
	KH	Be-42	Ishida	Nerima	Holte	Iowa-609
KH	640*	—	—	10	—	—
Be-42	10	6,320	140	—	10	—
Ishida	—**	5,120	450	20	60	—
Nerima	60	250	—	270	10	20
Holte	10	—	—	—	1,000	—
Iowa-609	80	—	—	—	—	380
A-5968	220	—	—	—	—	—
Connaught	40	100	—	—	—	10
IB-41	20	—	—	—	—	10
Shiga	20	—	—	—	—	—

*: $TCID_{50}$ determined in stationary tube cultures; expressed as reciprocal of the highest serum dilution causing 50 % reduction in appearing CPE.

**: Neutralizing-antibody titer was less than 1 : 10

Results

Cross-neutralization tests were performed with the 10 IBV strains and corresponding chicken sera. The results are shown in Table 1. No chicken injected with IB-41, Connaught, A-5968 or Shiga strain produced antibody against any of the 10 IBV strains tested. Though there were differences between homologous and heterologous titres, the antigenicities of all 10 IBV strains were related one to another. Particularly, anti-KH serum neutralized all heterologous strains except Ishida strain; all these reactions except that with the Nerima strain were one-way. Anti-Be-42 serum neutralized Ishida, Nerima and Connaught strains, especially Ishida strain was neutralized to the same extent as the homologous virus; Be-42 strain was neutralized by anti-KH, -Ishida and -Holte sera. Anti-Ishida serum neutralized only Be-42 strain; Ishida strain was neutralized by anti-Be-42, -Nerima and -Holte sera. Anti-Nerima serum neutralized KH and Ishida strains; Nerima strain was neutralized by all the antisera except anti-Ishida serum. Anti-Holte serum neutralized Be-42, Ishida and Nerima strains; Holte strain was neutralized by anti-KH serum. Anti-Iowa-609 serum neutralized Nerima, Connaught and IB-41 strains; Iowa-609 strain was neutralized by anti-KH serum.

The same results expressed as percentage of cross-neutralizing-titres ratio are shown in Table 2. If a tentative rough division into four groups is adopted, namely "homologous" cross-reaction with 100—25 % cross-neutralizing titre, "heterologous, highly" cross-reaction with 24—5 %, "heterologous, partially" cross-reaction with 4—1 %, and "no cross" reaction with less than 1 %, it is evident that most positive reactions are partially- or highly--crossed heterologous ones. Homologous cross-reaction was observed only between Be-42 and Ishida strains.

Next, cross-neutralization tests using 10 IBV strains and their specific rabbit sera were performed. The results obtained are shown in Table 3 and in Table 4 they are expressed in percentage of cross-neutralizing titre ratios. All strains except A-5968 cross-reacted with certain other strains, though most positive reactions revealed one-way relationship. All four IBV strains

Table 2. Data from Table 1 expressed as percentage of the cross-neutralization titre ratios

Virus strain	Antisera					
	KH	Be-42	Ishida	Nerima	Holte	Iowa-609
KH	100	—	—	4	—	—
Be-42	2	100	31	—	1	—
Ishida	—*	81	100	7	6	—
Nerima	9	4	—	100	1	5
Holte	2	—	—	—	100	—
Iowa-609	13	—	—	—	—	100
A-5968	34	—	—	—	—	—
Connaught	6	2	—	—	—	3
IB-41	3	—	—	—	—	3
Shiga	3	—	—	—	—	—

*: Neutralizing-antibody ratio was less than 1 : 100.

Table 3. Cross-neutralization tests among 10 IBV strains and corresponding rabbit sera

Virus strain	Antisera									
	KH	Be-42	Ishida	Nerima	Holte	Iowa-609	A-5968	Connaught	IB-41	Shiga
KH	320**	—**	—	—	80	—	—	—	—	—
Be-42	20	4,260	—	—	—	10	—	—	450	25
Ishida	—	1,260	640	100	10	—	—	10	—	—
Nerima	—	—	—	460	—	—	—	—	—	—
Holte	—	—	—	—	2,150	10	—	10	20	—
Iowa-609	—	—	—	—	20	6,310	—	—	—	—
A-5968	—	—	—	—	—	—	3,550	—	—	—
Connaught	—	—	—	—	10	—	—	640	20	190
IB-41	—	—	—	—	—	—	—	10	2,140	10
Shiga	—	—	—	—	—	—	—	10	—	460

*: $TCID_{50}$ determined in stationary tube cultures; expresed as reciprocal of the highest serum dilution causing 50 % reduction in CPE.
**: Neutralizing-antibody titre was less than 1 : 10.

Table 4. Data of Table 3 expressed as percentage of the cross-neutralization titre ratios

Virus strain	Antisera									
	KH	Be-42	Ishida	Nerima	Holte	Iowa-609	A-5968	Connaught	IB-41	Shiga
KH	100	—	—	—	4	—	—	—	—	—
Be-42	6	100	—	—	—	—	—	—	21	5
Ishida	—*	30	100	22	—	—	—	2	—	—
Nerima	—	—	—	100	—	—	—	—	—	—
Holte	—	—	—	—	100	—	—	2	1	—
Iowa-609	—	—	—	—	1	100	—	—	—	—
A-5968	—	—	—	—	—	—	100	—	—	—
Connaught	—	—	—	—	—	—	—	100	1	41
IB-41	—	—	—	—	—	—	—	2	100	2
Shiga	—	—	—	—	—	—	—	2	—	100

*: Neutralizing-antibody ratio was less than 1 : 100.

not producing antibody in chicken did so in rabbits. Connaught strain reacted with Holte, Ishida, IB-41 and Shiga strains; IB-41 strain with Be-42, Connaught and Shiga strains, Shiga strain with Be-42, Connaught and IB-41 strains; and as mentioned above, the A-5968 strain did not react with any other strain. On the other hand, cross-relationships among the 10 IBV strains seemed to be rather infrequent as compared to those observed with chicken sera. In rabbit serum, anti-KH serum neutralized only Be-42 strain; anti--Be-42 serum did not neutralize Nerima or Connaught strain; anti-Ishida serum did not neutralize Be-42 strain; anti-Nerima serum did not neutralize KH strain; anti-Holte serum did not neutralize Be-42 strain; anti-Iowa-609 serum did not neutralize strongly any other strains. Table 4 shows some serological relationships among all 10 IBV strains except A-5968 strain. It seems impossible to group serologically these IBV strains, because A-5968 reacted with anti-KH strain chick serum.

Discussion

SPF chickens have been used to prepare specific antiserum in most serological studies of IBV. Care must be always taken not to contaminate the chickens with another IBV or avian virus. In addition, chickens failed to produce antibody against such IBV strain which has been passed numerous times in ovo or in vitro. It was desirable to search for suitable animals producing specific antibody against IBV. There have been, however, only a few reports describing preparation of antiserum to IBV in mammals such as guinea-pigs (Kawamura *et al.*, 1968) and rabbits (Estola, 1966). Hofstad (1958) pointed that the rabbit was unsuitable for production of virus-neutralizing antibody, because some inhibitor-like substance against IBV is present in the serum of many rabbits and it is difficult to produce virus-neutralizing antibody against IBV. In the present study, however, no such inhibitor-like substance was found in any rabbit serum and IBV-injected rabbits produced virus-neutralizing antibody against all homologous IBV strains. A slight difference was observed, however, in cross-reactions between chicken and rabbit sera. Heterologous reactions with chicken sera were observed more frequently than with rabbit sera. These results indicate that rabbits showed less sensitive immune-response against IBV than chickens did. Chickens may be more suitable than rabbits to prepare the antibody against IBV particles as pointed out by Hofstad (1958). Yet, it has to be noticed that preparing specific immune serum against IBV strain that had been passaged numerous times in ovo or in vitro was difficult. In the present investigation, it was presumed that all the IBV strains tested belong serologically to a mono-type as pointed out by Cowen and Hitchner (1975). These strains cannot be arranged into different serotypes, though most cross-relationships among these IBV strains showed heterologous cross and one-way reaction, and only few a homologous cross-relationship.

As stated above, a number of so-called "serotypes" of IBV have been reported by virus-neutralization tests (Cumming, 1963; Hopkins, 1969;

Cowen *et al.*, 1971; Winterfield *et al.*, 1971; Johnson *et al.*, 1973, 1976; Raggi and Gomez, 1975; Johnson and Marquardt, 1976). No extensive study has been made to estimate the number of "serotypes" and on their true nature because such task would be possibly laborious. In 1972, WHO/FAO working team on coronaviruses proposed that IBV should be grouped into serotypes by the same procedure, and nomenclature for prototypes of IBV has been proposed: 1, Massachusetts; 2, Connecticut and 3, other candidates that will have to be determined (Cunningham, 1973). In the present study, serological relationship between KH strain belonging to Massachusetts type (Kawamura *et al.*, 1968) and A-5968 strain belonging to Connecticut type (Hofstad, 1958) was demonstrated. Strange enough, there was a slight reaction among Massachusetts-type strains as IB-41, Be-42, Connaught and KH strains; these phenomena had already been pointed out by Chomiak *et al.* (1963) and Hopkins (1974). Furthermore, in some protection tests for evaluation of IBV live vaccine, a broad spectrum of protection from developing respiratory signs was observed (Rosenberger *et al.*, 1976; Winterfield *et al.*, 1976); similar results were obtained by the immunofluorescent antibody reactions (Lukert, 1969). On the other hand, differences in antigenic constitution of the 10 IBV strains seems to be more striking than that of type 2 polioviruses (Wenner *et al.*, 1956). Such differences may have caused numerous so-called "serotypes"; no criterion for assessing cross-reaction had been established.

We have previously demonstrated (Otsuki *et al.*, 1982) that IBV changed its antigenicity during passages in BHK-21 cells. Be-42 strain 50-times passaged reacted weakly with antiserum to the parent virus, that is, virus-neutralizing antibody titre of parent virus was 1 : 725 but that of BHK-21-cell passaged virus was only 1 : 20. Therefore, it is presumed that antigenic determinants of IBV are fairly homogenous but antigenic drift occurs rather easily like in togaviruses (Henderson and Hoshino, 1970), transmissible gastroenteritis virus of swine (Morilla and Ristic, 1973), equine infectious anemia virus (Kono et al., 1973), visna virus (Narayan *et. al.*, 1977), rabies virus (Wiktor and Koprowski, 1980) and poliovirus (Gard, 1960). For future, it might be interesting to use monoclonal antibodies for differentiation of IBV strains.

References

Chomiak, T. W., Luginbuhl, R. E., and Steele, F. M. (1963): Serologic differences between the Beaudette and Massachusetts strains of infectious bronchitis virus. *Avian Dis.* **7**, 325–330.

Cowen, B. S., and Hitchner, S. B. (1975): Serotyping of avian infectious bronchitis viruses by the virus-neutralization test. *Avian Dis.* **19**, 583–595.

Cowen, B. S., Hitchner, S. B., and Lucio, B. (1971): Characterization of a new infectious bronchitis virus isolate. I. Serological and pathogenicity studies of Clark 333. *Avian Dis.* **15**, 518–526.

Cumming, R. B. (1963): Infectious bronchitis (uremia) in Australia. *Aust. Vet. Sci.* **39**, 115–147.

Cunningham, C. H. (1973): Immunologic methods in avian research: neutralization test. *Avian Dis.* **17**, 227–235.

Doi, M., Yamakami, T., Koimaru, H., Yoshimura, M., Masu, S., Shirai, J., and Kawamura, H. (1982): Serotypes of avian infectious bronchitis virus isolates from field cases in Japan. *Avian Dis.* **26**, 946–956.

Estola, T. (1966): Studies on the infectious bronchitis virus of chickens isolated in Finland. *Acta Vet. Scand.* **18**, 1–111 (supplement).

Hitchner,S. B., Appleton, G. S., and Winterfield, R. W. (1964): Evaluation of the immunity response to infectious bronchitis virus. *Avian Dis.* **8**, 153—162.

Hofstad, M. S. (1958): Antigenic differences among isolates of avian infectious bronchitis virus. *Am. J. vet. Res.* **19**, 740—743.

Hopkins, S. R. (1969): Serologic and immunologic properties of a recent isolate of infectious bronchitis virus. *Avian Dis.* **13**, 356—362.

Hopkins, S. R. (1974): Serological comparisons of strains of infectious bronchitis virus using plaque-purified isolants. *Avian Dis.* **18**, 231—239.

Johnson, R. B., and Marquardt, W. W. (1975): The neutralizing characteristics of strains of infectious bronchitis virus as measured by the constant-virus variable-serum method in chicken tracheal cultures. *Avian Dis.* **19**, 82—90.

Johnson, R. B., and Marquardt, W. W. (1976): Strains of infectious bronchitis virus on the Delmarva Peninsula and in Arkansas. *Avian Dis.* **20**, 382—386.

Johnson, R. B., Marquardt, W. W., and Newman, J. A. (1973): A new serotype of infectious bronchitis virus responsible for respiratory disease in Arkansas broiler flocks. *Avian Dis.* **17**, 518—523.

Johnson, R. B., Marquardt, W. W., Eskelund, K. H., and Gerencer, W. (1976): New strains of infectious bronchitis virus isolated in Maine. *Avian Dis.* **20**, 173—178.

Jungherr, E. L., Chomiak, T. W., and Luginbuhl, R. E. (1956): Immunologic differences in strains of infectious bronchitis, pp. 203—209. Proc. 60th Ann. Meet. U.S. Livestock Sanit. Assoc.

Kawamura, H., Shimizu, F., Yuasa, N., and Tsubahara, H. (1968): Infectious bronchitis virus type-incidence in Japan. Jap. *J. Vet. Sci.* **30**, 321 (supplement).

Kono, Y., Kobayashi, K., and Fukunaga, Y. (1973): Antigenic drift of equine infectious anemia virus in chronically infected horses. *Arch. ges. Virusforsch.* **41**, 1—10.

Lukert, P. D. (1969): Differentiation of avian infectious bronchitis virus serotypes by immunofluorescence. *Avian Dis.* **13**, 847—852.

Morilla, A., and Ristic, M. (1973): Immunologic discrepancy between intestinal and cell culture viruses of transmissible gastroenteritis of swine. *Am. J. Vet. Res.* **34**, 1533—1538.

Narayan, O., Criffin, D. E., and Chase, J. (1977): Antigenic shift of visna virus in persistently infected sheep. *Science* **197**, 376—378.

Otsuki, K., Tagawa, Y., and Tsubokura, M. (1982): Antigenic variation of avian infectious bronchitis virus during replication in BHK-21 cells. *Arch. Virol.* **73**, 75—78.

Otsuki, K., Yamamoto, H., and Tsubokura, M. (1979*a*): Studies on avian infectious bronchitis virus (IBV). I. Resistance of IBV to chemical and physical treatments. *Arch Virol.* **60**, 25—32.

Otsuki, K., Noro, K., Yamamoto, H., and Tsubokura, M. (1979*b*): Studies on avian infectious bronchitis virus (IBV). II. Propagation of IBV in several cultured cells. *Arch. Virol.* **60**, 115—122.

Otsuki, K., Maeda, J., Yamamoto, H., and Tsubokura, M. (1979*c*): Studies on avian infectious bronchitis virus (IBV). III. Interferon induction by and sensitivity to interferon of IBV. *Arch. Virol.* **60**, 249—255.

Raggi, L. G., and Gomez, L. (1975): Two new isolants of infectious bronchitis virus with polyvalent immunogenicity. *Avian Dis.* **19**, 323—333.

Reed, L. J., and Muench, H. (1938): A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.* **27**, 493—497.

Rosenberger, J. K., Appleton, R. L., and Kraus, W. C. (1976): Cross-protection studies with a Holland strain (Noblis H-52) of infectious bronchitis virus. *Avian Dis.* **20**, 199—201.

Wenner, H. A., Kamitsuka, P., and Lenahan, M. (1956): A comparative study of type 2 poliomyelitis viruses. II. Antigenic differences relating to 18 type 2 strains. *J. Immunol.* **77**, 220—231.

Wiktor, T. J., and Koprowski, H. (1980): Antigenic variants of rabies virus. *J. exp. Med.* **152**, 99—112.

Winterfield, R. W., and Hitchner, S. B. (1962): Etiology of an infectious nephritis-nephrosis syndrome of chickens. *Am. J. vet. Res.* **23**, 1273—1279.

Winterfield, R. W., Fadly, A. M., and Hanley, J. E. (1971): Characteristics of an isolate of infectious bronchitis virus from chickens in Florida. *Avian Dis.* **15**, 305—311.

Winterfield, R. W., Fadly, A. M., and Hoerr, F. J. (1976): Vaccination and revaccination with a Holland (H) strain of infectious bronchitis virus. *Avian Dis.* **20**, 369—374.