Studies on Avian Infectious Bronchitis Virus (IBV)

I. Resistance of IBV to Chemical and Physical Treatments

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With 2 Figures

Accepted November 23, 1978

Summary

The resistance of avian infectious bronchitis virus (IBV) to several chemical and physical treatments was studied. Ten strains, including four Japanese strains, were used.

1. All strains were sensitive to heating at 56° C for 15 minutes; although two of them, KH and Massachusetts-41, were resistant to heating at 45° C for 90 minutes. 2. All strains were resistant to pH 3.0 and most of the strains were sensitive to pH 11.0. 3. All strains were completely inactivated by chloroform and sodium deoxycholate and all except Beaudette-42 and Connaught were relatively stable to ether. 4. All strains rapidly lost their infectivities upon ultraviolet irradiation. 5. Trypsin did not affect the infectivity of any strain. 6. From these results, the ten strains were classified into three groups based on their stabilities to exposure to heating at 45° C for 90 minutes and to ether.

Introduction

Avian infectious bronchitis virus (IBV) is the most intensively studied coronavirus. The sensitivities of some IBV strains, for example Beaudette, Massachusetts, and Connecticut, to chemical and physical treatments have been thoroughly examined (4, 6, 13, 14, 17, 21, 22, 23), but the results have differed from one report to another. Contradictory results have been particularly reported on the resistance of the strains to exposure to 56° C (6, 9, 13, 17, 18, 22), to pH 3.0 (3, 4, 7, 9, 12, 18, 20), to pH 12.0 (18, 20), to trypsin (4, 5, 9, 17, 21, 22), and to ether (9, 12, 14, 18, 20). Many other strains have not been examined.

Since the antigenic structure of IBV has not been clarified, the identification of IBV strains is mainly derived from their morphological, chemical and physical characteristics. Thus, it is very important to examine the properties of IBV. In the present investigation, we examined ten typical strains of IBV, including four Japanese strains, for their resistance to various chemical and physical agents.

Materials and Methods

Virus Strains

The ten strains of IBV examined were: Beaudette-42 (Be-42), Massachusetts-41 (IB-41), Connecticut A-5968 (A-5968), Connaught, Holte, Iowa-609, KH, Nerima, Ishida, and Shiga. The last four strains were isolated in Japan. All strains were kindly supplied by the National Institute of Animal Health, Tokyo (Director Dr. S. Shibata). The strains had been passaged over 15 times in chick embryo and from 4 (Ishida strain) to 122 (KH strain) times in cultured chicken kidney (CK) cells before they were analysed in this laboratory. All the strains were shown, in this laboratory, to produce a cytopathic effect (CPE) in primary CK cells, and they were all cloned two or three times in CK cells prior to examination.

Cell Culture

Primary kidney cells from 2- to 3-week-old chickens were cultured as reported previously (15).

Virus Titration

A ten-fold dilution of each treated virus preparation was made in Hanks' balanced salt solution and a 0.1-ml aliquot was inoculated into each of four or five tubes of CK cells. The virus was adsorbed on to the cell sheet for 90 minutes at 37° C and then a 0.6-ml portion of maintenance medium was added to each tube. The TCID₅₀ titer was calculated as described by REED and MUENCH (19).

Heat Stability

The heat stability of IBV was examined using the method of HOFSTAD (13). Tubes containing a 20-fold dilution of virus-infected culture medium in sterile distilled water were placed in a water-bath at 56°, 45°, or 40° C. At 5-minute intervals, tubes were removed and immediately put in an ice-bath. The amount of virus surviving was then titrated.

pH Stability

Virus culture medium was adjusted to pH 3.0 or pH 11.0 by adding 0.1 N HCl or 0.1 N NaOH, and was then dispensed into small tubes at 4° C. After 180 minutes the pH of the tubes was adjusted to 7.2 with 0.1 N NaOH or 0.1 N HCl, and the virus viability was assayed. Control virus was kept at pH 7.2.

Sensitivity to Lipid Solvents

The sensitivity of IBV to lipid solvents was determined as described previously for ether (1), chloroform (10) and sodium deoxycholate (16).

Sensitivity to Trypsin

Trypsinization of IBV was performed as follows. One milliliter of virus culture medium, 13 ml of sterile distilled water and 2 ml of 2 per cent trypsin (DIFCO, 1:250) were put into each test tube. One milliliter of virus culture medium and 15 ml of sterile distilled water were put into each control tube. Test and control tubes were divided into two groups; one group was incubated at room temperature and the other at 37° C. After 6 hours 4 ml of a 1 per cent trypsin inhibitor (MILES) solution was added to the test tubes and 4 ml of sterile distilled water to the control tubes. The tubes were titrated for virus viability. The sensitivity of IBV to trypsin was judged by comparing the virus titers of the test and control tubes.

Sensitivity to Ultraviolet (UV) Irradiation

A 10 ml portion of virus culture medium in a petri dish (6 cm in diameter) was irradiated for 20 seconds with a UV lamp (15 W, 0.5 A) at a distance of 80-cm. Virus viability was assayed after irradiation.

Results

Resistance to Heat

All strains of IBV were rapidly inactivated on exposure to 56° C (Fig. 1). Virus viability was reduced in most strains by 4 log₁₀ or more in 15 minutes. Figure 2 shows the resistance to exposure to 45° C for the IBV strains. The titers of the IB-41 and KH strains were reduced by less than 1 log₁₀ in 90 minutes. However, greatly decreased infectivities were observed after 30 minutes and no infectious virus were observed after 90 minutes, with the other eight IBV strains.

Fig. 1. Sensitivity of IBV strains to 56° C. Initial virus titer of Be-42 strain was $10^{5.6}$ TCID₅₀, IB-41 $10^{5.8}$, A-5968 $10^{5.8}$, Connaught $10^{6.0}$, Holte $10^{5.6}$, Iowa-609 $10^{5.3}$, KH $10^{4.8}$, Nerima $10^{4.5}$, Ishida $10^{4.3}$, and Shiga $10^{4.3}$.

Fig. 2. Sensitivity of IBV strains to 45° C. Initial virus titer of Be-42 strain was $10^{6.0}$ TCID₅₀/0.1 ml, IB-41 $10^{5.8}$, A-5968 $10^{5.6}$, Connaught $10^{5.8}$, Holte $10^{5.0}$, Iowa-609 $10^{5.3}$, KH $10^{4.8}$, Nerima $10^{4.5}$, Ishida $10^{5.8}$, and Shiga $10^{5.0}$.

Resistance to Acid and Alkali

The results are shown in Table 1. All the strains were relatively resistant to pH 3.0 treatment. However, most strains were more inactivated on exposure to pH 11.0. Here the decrease in the infectivity titer of the A-5968 strain was $1.5 \log_{10}$ in 120 minutes, while the other strains shows decreases in their infectivity titers of 3.3 to $4.2 \log_{10}$ in 120 minutes. Thus, only the A-5968 strain was regarded as stable at pH 11.0.

Sensitivity to Lipid Solvents

The results are summarized in Table 2. No viable virus from any strain was detected after exposure to chloroform or sodium deoxycholate. On the other hand, all the strains except Be-42 and Connaught retained some activity after exposure to ether, although their titers decreased by 2.0 to $3.8 \log_{10}$. The strains showing some activity after exposure to ether were regarded as ether-stable.

Table 1. *pH stability tests on IBV strains*

Virus strain	Virus titer ^a reduction at	
	pH 3.0	pH 11.0
Be-42	1.0	3.4
IB-41	1.5	4.0
A-5968	1.0	1.5
Connaught	1.5	4.0
Holte	0.3	4.2
Iowa-609	0.2	3.8
KH	0.3	4.0
Nerima	0.2	4.0
Ishida	2.0	4.0
Shiga	1.0	3.3

^a $\log_{10}$ -TCID₅₀

Table 2. *Sensitivity of IBV strains to lipid solvents*

Virus strain	Initial	Without solvent	Ether	Chloroform	Sodium
		(4° C for 18 hours)	(20%, 4° C for 18 hours)	(50%, r. t. ^b for 10 min-utes)	deoxycholate (0.1%, 4° C for 18 hours)
Be-42	6.2	5.0	NS ^a	NS	NS
IB-41	6.5	6.4	3.7	NS	NS
A-5968	5.5	5.2	1.4	NS	NS
Connaught	5.2	4.2	NS	NS	NS
Holte	6.7	6.4	4.4	NS	NS
Iowa-609	6.2	4.4	2.4	NS	NS
KH	6.5	6.2	3.4	NS	NS
Nerima	7.2	5.9	2.9	NS	NS
Ishida	6.5	5.2	2.4	NS	NS
Shiga	5.8	4.0	1.4	NS	NS

The table indicates $\log_{10}$ -TCID₅₀/0.1 ml

^a NS: No survival of virus

^b r. t.: Room temperature

Sensitivity to Trypsin

No significant difference in the titer between trypsinized and control viruses was observed after 6 hours at room temperature (Table 3). Furthermore, none of the strains was affected markedly on trypsinization at 37° C. These results show that these IBV strains are resistant to trypsin.

Sensitivity to UV Irradiation

All strains were highly sensitive to UV irradiation. Their infectivities rapidly decreased on irradiation for 20 seconds from a distance of 80 cm. There were no marked differences in UV sensitivity between the strains.

Table 3. *Sensitivity of IBV strains to 0.25 per cent trypsin*

Virus strain	Initial	Virus titer ^a			
		37° C		Room temperature	
		Trypsin	Without trypsin	Trypsin	Without trypsin
Be-42	6.5	1.5	2.5	3.0	3.8
IB-41	4.8	2.0	2.8	3.7	3.7
A-5968	6.0	2.7	3.0	4.7	4.3
Connaught	5.8	3.0	3.0	3.3	3.5
Holte	5.3	1.5	2.8	3.5	3.8
Iowa-609	4.5	1.3	2.0	2.5	3.0
KH	5.8	2.7	3.8	4.5	4.5
Nerima	5.3	2.5	2.8	3.3	3.5
Ishida	5.5	1.8	2.3	3.0	3.5
Shiga	5.5	1.3	1.3	3.7	3.7

^a log₁₀-TCID₅₀/0.1 ml

Table 4. *Summary of the results of sensitivities of IBV strains to chemical and physical agents*

Exposure to	Virus strain									
	Be-42	IB-41	A-5968	Con-naught	Holte	Iowa-609	KH	Nerima	Ishida	Shiga
56° C,	— ^a	—	—	—	—	—	—	—	—	—
15 minutes										
45° C,	—	+ ^b	—	—	—	—	+	—	—	—
90 minutes										
pH 3.0	+	+	+	+	+	+	+	+	+	+
pH 11.0	—	—	+	—	—	—	—	—	—	—
Ether	—	+	+	—	+	+	+	+	+	+
Chloroform	—	—	—	—	—	—	—	—	—	—
Sodium deoxycholate	—	—	—	—	—	—	—	—	—	—
Trypsin	+	+	+	+	+	+	+	+	+	+
UV	—	—	—	—	—	—	—	—	—	—

^a — : Sensitive

^b + : Resistant

Summary of the Sensitivities of IBV to Chemical and Physical Treatments

The results of the different treatments are summarized in Table 4. All strains were sensitive to heating at 56° C for 15 minutes, pH 11.0 (although the A-5968 strain was partially resistant), and to treatment with chloroform, sodium deoxycholate and UV irradiation. All strains were resistant to pH 3.0, and to trypsin treatment. On the other hand, resistance to heating at 45° C for 90 minutes, and to ether treatment varied with each strain. The ten strains tested can be classified into three groups on the basis of their sensitivities to the last two treatments (Table 5). Group I includes the Be-42 and Connaught strains, which are sensitive to both treatments; group II includes six strains, which are relatively resistant to ether but sensitive to heating at 45° C for 90 minutes; and group III includes the IB-41 and KH strains, which are resistant to both treatments.

Table 5. *Grouping of IBV on the basis of their sensitivity to heating to 45° C and to ether treatment*

Treatment	Group		
	I	II	III
45° C, 90 minutes	— ^a	—	+ ^b
Ether	—	+	+

^a — : Sensitive

^b + : Resistant

Discussion

Although IBV is the most extensively studied coronavirus, the sensitivity of IBV strains to chemical and physical treatments has not been clarified. Thus, in the present investigation, ten IBV strains, including four Japanese strains, were examined for their sensitivities to various treatments.

CUNNINGHAM *et al.* (5, 17) have shown that trypsin inactivates several United States strains of IBV. However, the results obtained in the present investigation and those obtained by others (4, 9, 21, 22) show that most IBV strains are trypsin-resistant.

All the strains tested were sensitive to chloroform and sodium deoxycholate as shown by other investigators (8, 11, 17, 21) and this indicates that lipid is an essential component of IBV. Coronaviruses are defined as being ether sensitive (9, 12, 14, 20) although QUIROZ and HANSON (18) reported that IBV is relatively resistant to ether. In the present investigation, Be-42 and Connaught strains completely lost their infectivity on exposure to ether, whereas the other eight strains were relatively stable to ether treatment.

ESTOLA (9) regarded IBV as acid-labile, while others reported that IBV is acid-stable (4, 7, 12, 18, 20) or variable (3). Some difficulty has arisen in determining when IBV strains are acid-stable and this may have caused these inconsistent results. In fact, some reports have regarded IBV as acid-stable when only a small portion of virus particles survived exposure to pH 2 or 3 (12, 18, 20). In the present investigation, since the infectivity titers of all the strains were reduced by less than 2 log₁₀ on treatment at pH 3.0 for 6 hours at 4° C, we consider that these IBV strains are pH 3.0-resistant.

Only one thermostable IBV strain has been reported (13) and most IBV have been shown to be sensitive to heating (6, 9, 17, 18, 22). In this study all strains were sensitive to heating at 56° C and only two of the strains were resistant to heating at 45° C for 90 minutes. From these results, we consider that thermostability depends on the intrinsic characteristics of the IBV strain rather than the number of passages (13). The number of passages for the strains were different, and there was no relationship between resistance to heat and the number of passage.

No difference was observed between the strains with respect to resistance to 56° C, pH 3.0, pH 11.0 (except the A-5968 strain), chloroform, sodium deoxycholate, UV irradiation or trypsin.

IBV strains tested were classified into three groups, I, II and III, on the basis of their sensitivities to ether and to heating at 45° C for 90 minutes. Since IBV serotyping has not been established, a satisfactory classification method is necessary. The tentative grouping of the IBV strains described in this paper may be useful as a basis for the subdivision of IBV strains.

References

1. ANDREWS, C. H., HORSTMANN, D. M.: Susceptibility of viruses to ethyl ether. *J. gen. Microbiol.* **3**, 290—297 (1949).
2. COOPER, P. D.: A chemical basis for the classification of animal viruses. *Nature* **190**, 302—305 (1961).
3. COWEN, B. S., HITCHNER, S. B.: pH stability studies with avian infectious bronchitis virus (coronavirus) strains. *J. Virol.* **15**, 430—432 (1975).
4. COWEN, B. S., HITCHNER, S. B., UBERTINI, T.: Characterization of a new infectious bronchitis virus isolate II. Some chemical and physical properties of Clark 333. *Avian Dis.* **15**, 527—532 (1971).
5. CUNNINGHAM, C. H.: Avian infectious bronchitis. In: BRANDLY, C. A., CORNELIUS, C. E. (eds.), *Advances in veterinary science and comparative medicine*, Vol. 14, 105—148. New York: Academic Press 1970.
6. CUNNINGHAM, C. H., SPRING, M. P.: Some studies of infectious bronchitis virus in cell culture. *Avian Dis.* **9**, 182—193 (1965).
7. CUNNINGHAM, C. H., STUART, H. O.: The pH stability of the virus of infectious bronchitis of chickens. *Cornell Vet.* **37**, 99—103 (1947).
8. DuBOSE, R. T., GRUMBLES, L. C., FLOWERS, A. I.: Differentiation of quail bronchitis virus and infectious bronchitis virus by heat stability. *Amer. J. vet. Res.* **21**, 740—743 (1960).
9. ESTOLA, T.: Studies on the infectious bronchitis virus of chickens isolated in Finland. *Acta vet. Scand.* **18**, 1—111, supplement (1966).
10. FELDMAN, H. A., WANG, S. S.: Sensitivity of various viruses to chloroform. *Proc. Soc. exp. Biol. (N.Y.)* **106**, 736—738 (1961).
11. HAMRE, D., PROCKNOW, J. J.: A new virus isolated from the human respiratory tract. *Proc. Soc. exp. Biol. (N.Y.)* **121**, 190—193 (1966).
12. HIRAI, K., SHIMAKURA, S.: Isolation and characteristics of avian nephrosis-inducing infectious bronchitis virus (coronavirus). *Jap. J. vet. Sci.* **33**, 209—216 (1971).
13. HOFSTAD, M. S.: Stability of avian infectious bronchitis virus at 56° C. *Cornell Vet.* **46**, 122—128 (1956).
14. MOHANTY, S. B., CHANG, S. C.: Development and ether sensitivity of infectious bronchitis virus of chickens in cell cultures. *Amer. J. vet. Res.* **24**, 822—826 (1963).
15. OTSUKI, K., TAKAHASHI, K., TSUBOKURA, M., ITAGAKI, K., NISHIO, S.: Detection of neutralizing antibodies using several serological types of avian infectious bronchitis virus in a poultry farming area. *Japan. Poult. Sci.* **13**, 20—25 (1976).

16. OTSUKI, K., TSUBOKURA, M., YAMAMOTO, H., IMAMURA, M., SAKAGAMI, Y., SAIO, H., HOSOKAWA, D.: Some properties of avian adenoviruses isolated from chickens with inclusion body hepatitis in Japan. *Avian Dis.* **20**, 694—705 (1976).
17. PAGE, C. A., CUNNINGHAM, C. H.: The neutralization test for infectious bronchitis virus. *Amer. J. vet. Res.* **23**, 1065—1071 (1962).
18. QUIROZ, C. A., HANSON, R. P.: Physical-chemical treatment of inocula as a means of separating and identifying avian viruses. *Avian Dis.* **2**, 94—98 (1958).
19. REED, L. J., MUENCH, H. A.: Simple method of estimating fifty percent endpoints. *Amer. J. Hyg.* **27**, 493—497 (1938).
20. SINGH, V. P., MALIK, B. S., VERMA, K. C.: Physical and chemical characteristics of a local strain of infectious bronchitis virus IBV of poultry. *Indian J. Poult. Sci.* **9**, 168—171 (1974).
21. STEELE, F. M., LUGINBUHL, R. E.: Direct and indirect complement-fixation tests for infectious bronchitis virus. *Amer. J. vet. Res.* **25**, 1249—1255 (1964).
22. VON BÜLOW, V.: Infektiöse Bronchitis der Hühner IV. Charakterisierung eines neuen Feldstammes des IB-Virus (IBV-10). *Zbl. Vet.-Med.* **14**, 151—162 (1967).
23. WINTERFIELD, R. W., FADLY, A. M., HANLEY, J. E.: Characteristics of an isolate of infectious bronchitis virus from chickens in Florida. *Avian Dis.* **15**, 305—311 (1971).

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Received June 5, 1978