SELECTION OF VARIANTS OF AVIAN INFECTIOUS BRONCHITIS VIRUS SHOWING TROPISM FOR DIFFERENT ORGANS

Koichi Otsuki, Kohei Matsuo, Nobuyuki Maeda,
Takeshi Sanekata, Misao Tsubokura

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

ABSTRACT

Avian infectious bronchitis virus strain Kagoshima-34 isolated from the kidneys of a chicken that died of nephrosis/nephritis lost its nephropathogenicity during intratracheal passage in SPF chickens. The resultant virus acquired stronger respirotropism but reduced tropism for kidneys. On the other hand strain Tottori-2 isolated from the trachea of a chicken suffering from severe respiratory disease did not lose its respirotropism after serial intravenous passage in SPF chickens.

The serological properties of the passaged virus were investigated by virus neutralisation test. The antibody titres of both strains of virus fluctuated with progressive passage. The serological properties of the virus isolated from respiratory organs were not necessarily the same as those of the isolates made from the kidneys.

INTRODUCTION

Avian infectious bronchitis virus (IBV) grows not only in respiratory organs of chickens but also in the kidneys[1,2,5]. IBVs causing respiratory disease have strong respirotropism and those causing nephrosis/nephritis have nephrotropism[5]. We had already shown experimentally that IBV changed its serological properties after serial passages in cultured mammalian cells [3]. It is well known that most strains of IBV lose their virulence for chickens following repeated passage in vitro or in ovo. It is possible that IBV changes its properties including its organ tropism with ease. We have tried to induce IBV to alter its organ tropism.

MATERIALS AND METHODS

Virus. Two Japanese isolates were tested in this investigation. Strain Kagoshima-34, isolated from the kidneys of a chicken that died of nephrosis/nephritis and strain Tottori-2, isolated from the trachea of a chicken showing severe respiratory signs[5]. Neither virus caused any CPE in cultivated chick kidney (CK) cells, but both strains caused curling or dwarfing of hen's embryos.

Virus titration. Virus was titrated as described previously[5].

Virus neutralisation test. Immune sera were prepared according to the method described previously[4]. Virus neutralisation test was carried out

Coronaviruses and Their Diseases
Edited by D. Cavanagh and T.D.K. Brown
Plenum Press, New York, 1990

in 8-day-old hen's eggs by the constant-serum varying-virus method[5].

In vivo passage of IBVs.

Intravenous passage. With both strains, virus at a titre of $10^{6.5}$ EID_{50} was injected into the ulvar cutaneous vein of two 6-week-old SPF chickens. The chickens were killed 4 days postinoculation (p.i.). Kidneys and such respiratory organs as trachea and lungs were taken aseptically, homogenised respectively with sterile sand and suspended to approximately 10% in Eagle's MEM. After clarification, portions of the supernatant of the kidneys were injected into the ulvar cutaneous vein of another two SPF chickens. Both IBV strains were passaged in this way ten times.

Intratracheal passage. With both strains, virus at a titre of $10^{6.5}$ EID_{50} was inoculated intratracheally into two 6-week-old SPF chickens. Trachea, lungs and kidneys were taken 3 or 4 days p.i., the former two tissues were pooled and homogenised. After clarification supernatant of the respiratory suspension was inoculated intratracheally another two SPF chickens. IBV was passaged ten times in the same manner.

Experimental infection with IBV. For each strain, 12 10-week-old SPF chickens were inoculated with $10^{6.0}$ EID_{50} of virus intratracheally. Two chickens were killed at 1, 2, 3, 5, 7 and 10 days p.i.. Trachea, lungs and kidneys were taken aseptically and the former two tissues were pooled and virus recovery attempted as described previously[5].

RESULTS

Organ tropism of passaged IBV

Initially, we tried to passage both strains of IBV by intratracheal inoculation. Strain Kagoshima-34 was passed ten times via the respiratory tract of SPF chickens. As shown in Table 1, this virus altered its organ tropism during passage. After 10 passages the virus caused only mild respiratory disease and macroscopical lesions in respiratory organs. Neither diarrhoea nor macroscopical lesions were observed in the kidneys, although considerable amounts of virus were still recovered from that tissue.

Table 1. Pathogenicity of strain Kagoshima-34 passaged in the respiratory organs

Passage number	Virus titre recovered from		Respiratory signs	Diarrhoea	Macroscopical change in	
	R.O.*	Kidneys			R.O.	Kidneys
1	2.3**	1.2	+ +	+ +	+ +	+ +
2	4.5	3.3	+ +	+ +	+ +	+ +
3	5.5	2.8	+ +	+ +	+ +	- -
4	5.5	2.8	- -	- -	+ +	- -
5	5.5	2.7	- -	- -	- -	- -
6	4.1	3.5	- -	- -	- -	- +
7	5.0	4.0	- -	- -	- -	- +
8	3.3	4.0	- -	- -	- -	- -
9	5.5	3.0	+ +	- -	- -	- +
10	4.4	4.0	+ +	- -	+ +	- -

For Tables 1 and 2 *: respiratory organ +: positive
**: $\log_{10}$/0.2 g -: negative

Virus passaged in respiratory organs grew lesser in kidneys than did its parent virus, but grew better in respiratory organs than its parent virus (Fig. 1).

Strain Tottori-2 passaged in the same manner did not change its organ tropism but grew better than the parent virus in any target organs.

Next, we tried to pass both strains by intravenous injection. Strain Tottori-2 hardly survived ten passages in kidneys. There was no obvious difference in pathogenicity between passaged and parent virus (Table 2). Virus recovery of passaged virus was similar to that of its parent. On the other hand, strain Kagoshima-34 could not be passed more than five times in kidneys.

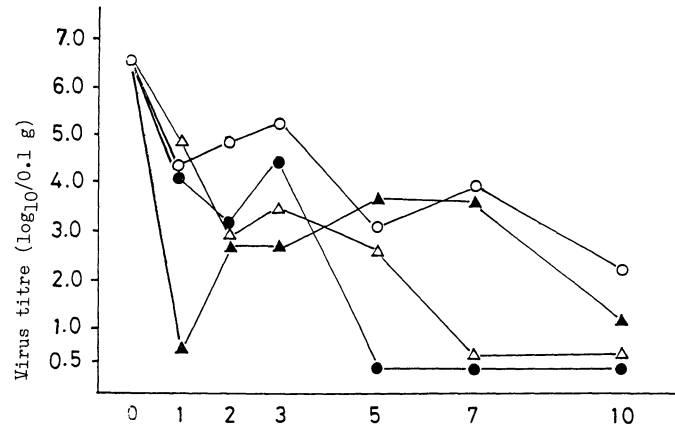

Fig. 1. Virus growth in chickens infected with strain Kagoshima-34. Comparison of the virus passed ten times in respiratory organs with the parent strain.
○——○ virus passaged from respiratory organs; ●——● parent virus
virus passaged from kidneys; △——△
from respiratory organs; ▲——▲ parent virus from kidneys.

Table 2. Pathogenicity of strain Tottori-2 passaged in the kidneys of chickens

Passage number	Virus titre recovered from R.O.*	Kidneys	Respiratory signs	Diarrhoea	Macroscopical change in R.O.	Kidneys
1	3.9**	3.0	- -	+ +	- -	- -
2	1.1	0.3	- -	- -	- -	- -
3	1.7	2.0	- -	- -	- -	- -
4	1.8	1.4	- -	+ -	- -	- -
5	1.6	1.8	- -	- -	- -	- -
6	5.2	2.8	- -	+ -	- -	- -
7	1.3	1.3	- -	+ +	- -	- -
8	1.8	3.0	- -	- -	- -	- -
9	1.0	2.7	- -	+ +	- -	- -
10	1.0	0.0	- -	- -	- -	- -

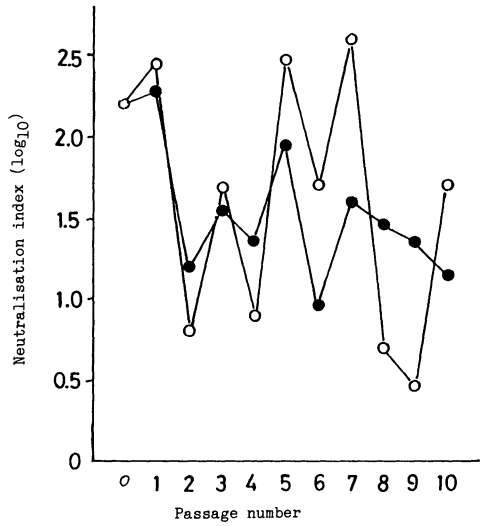

Fig. 2. Antigenicity of strain Tottori-2 passaged in respiratory organs. O——O virus recovered from respiratory organs; ●——● virus recovered from kidneys.

Antigenicity of passaged IBV

The antigenicity of IBV passaged in respiratory organs was tested by virus neutralisation test using virus recovered from the respiratory organs and kidneys respectively and hyperimmune sera against the parent virus. Fig. 2 shows that the antigenicity of Tottori-2 isolated from respiratory organs is not necessarily the same as that from kidneys even if they were recovered from the same chicken. The neutralising index of those viruses fluctuated with progressive passage, this was particularly so with virus recovered from the respiratory organs. Similar results were obtained with Kagoshima-34 (Fig. 3).

Resistance of passaged IBV to exposure to chemical and physical agents

Resistance of both strains of IBV passaged 3, 6, 8 and 10 times in respiratory organs of kidneys to exposure to pH 3.0 (4°C, 30 min), 5% chloroform (4°C, 20 min), 20% ethyl ether (4°C, 18 h) and heating (56°C, 15 min) was examined. All viruses were sensitive to all agents except to exposure to pH 3.0. These results are the same as those for the parent viruses.

DISCUSSION

IBV causing nephrosis/nephritis in chickens grows not only in the kidneys but also in the respiratory organs[1,2,5]. In this investigation, strain Kagoshima-34 isolated from the kidneys of a chicken that died of

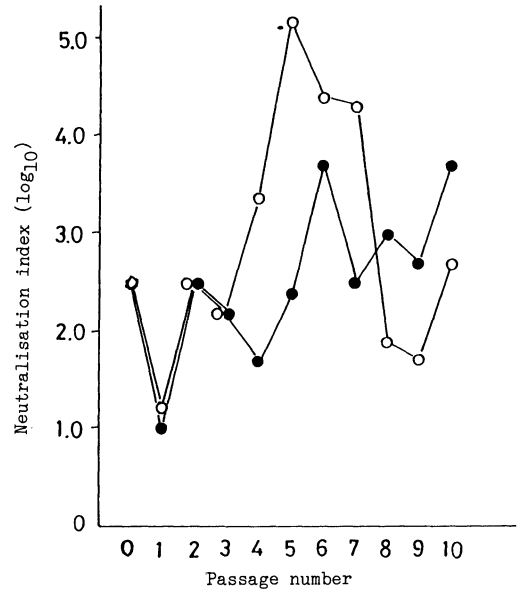

Fig. 3. Antigenicity of strain Kagoshima-34 passaged in respiratory organs. O——O Virus recovered from respiratory organs; ●——● Virus recovered from kidneys.

nephrosis/nephritis lost its nephropathogenicity following passage in respiratory organs of chickens but still caused respiratory disease. Strain Tottori-2 did not acquire obvious nephrotropism in spite of serial passages in kidneys. These results suggest that the target organ for IBV is not initially kidneys, but respiratory tissues.

With both virus strains, the antigenicity of the IBV recovered from kidneys was different from that recovered from the respiratory organs, even if both viruses were recovered from the same chicken. Antigenic alteration seemed to occur during passage regardless of whether virus was grown in respiratory organs or kidneys. In this investigation, it appears that alteration of organ tropism of IBV occurred unrelated to changes in antigenicity.

As we reported previously[3], an IBV strain seems to consist of various subpopulations. Thus it is possible that a subpopulation having respirotropism does not necessarily have nephrotropism. Both IBV strains tested here consist of considerably varied subpopulations and the antigenicity of these subpopulation varies.

Taguchi et al.[6] selected acute encephalitis-causing virus clone from mouse hepatitis virus JHM strain by intracerebral inoculation. They reported that the molecular size of a variant virus was larger than that of its parent virus.

Recently in Japan the incidence of nephrosis/nephritis caused by IBV is increasing even though several kinds of live IB vaccines have been widely used. It is possible that such vaccines caused selection of strongly nephrotropic IBV in the field, although we did not succeed in selecting nephrotropic virus clones from respiratory disease-causing IBV strains.

ACKNOWLEDGEMENTS

We are grateful to Dr Jane K. A. Cook for her advice in the preparation of this manuscript.

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