

Antigenic and immunogenic characterization of infectious bronchitis virus strains isolated in China between 1986 and 1995

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Eight strains of infectious bronchitis virus (IBV) were isolated between 1986 and 1995 from broilers and layers at eight different farms in four provinces in China. The viruses were isolated from flocks which suffered from either respiratory disease or nephritis and the majority had not been vaccinated against IBV. Six strains were shown by monoclonal antibodies to differ from H120, Connecticut and Arkansas 99 strains of IBV and also to differ from each other. Four of these strains were serotyped; one (NRZ) was of the Massachusetts serotype, three (HV, NB-90 and TJ) shared a degree of antigenic similarity and were of a serotype that differed from Massachusetts and Connecticut. NB-90 was similar to both Gray and T strains whereas TJ shared some similarity with the T strain. Four strains, HV, NB-90, YY and TJ induced 33, 47, 60 and 90% mortality, respectively, in 3-week-old specific pathogen-free chickens. Clinical signs and post-mortem findings were identical to those induced by the nephropathogenic T strain. Chickens vaccinated with H120 strain, and then challenged with four highly pathogenic strains HV, NB-90, YY and TJ were not protected as determined by both virus isolation and mortality. The results show that highly pathogenic IBV strains which induce clinical nephritis occur frequently in poultry flocks in China. They also confirm field observations on the lack of protection by currently used IB vaccines of the Massachusetts serotype against challenge with these nephropathogenic strains.

Introduction

Avian infectious bronchitis viruses (IBV) infect chickens of all ages causing significant economic losses to the poultry industry around the world. IBV causes respiratory disease, affects egg quality and production and induces significant mortality from nephritis (McMartin, 1993). Evidence to date shows that IBV can mutate readily and that strains may differ either antigenically, in pathogenicity or tissue tropism (Cook, 1984; King, 1988; Lucio & Fabricant, 1990; Gelb *et al.*, 1991; Cubillos *et al.*, 1991). Strains that differ in serotype are isolated frequently. The majority of these cause either respiratory disease in broilers or egg production problem in layers (Davelaar *et al.*, 1984; Cook & Huggins, 1986; El-Houadfi *et al.*, 1986; Avelaneda *et al.*, 1994). In comparison, nephropathogenic IBV strains that cause significant mortality have been isolated less frequently, Australia possibly being an exception to this trend (McMartin,

1993). During the last decade, however, nephropathogenic strains in IB-vaccinated flocks has been reported in Belgium, Italy, France and USA (Meulemans *et al.*, 1987; Zanella, 1988; Kinde *et al.*, 1991; Picault *et al.*, 1991; Lambrechts *et al.*, 1993).

Live vaccines based on Massachusetts strains have been used successfully throughout China for many years, H120 usually being administered from 1 to 10 days of age and H52 from 3 to 4 weeks of age. More recently, however, there have been widespread reports of disease resembling IBV nephritis in pullets and broilers, with 10 to 30% mortality in both non-vaccinated and vaccinated flocks. To date, only a limited characterization of IBV strains isolated from such cases has been reported (Zhang *et al.*, 1995; Wang *et al.*, 1997). It further has remained unclear to what degree currently used H120 and H52 vaccines are effective in controlling field infections, and whether vacci-

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nation failures are due to antigenic variation or inadequate vaccination.

In the present studies we aimed to examine the antigenic and protective relationship of IBV isolates and the H120 vaccine, as well as to compare antigenic properties and pathogenicity of these isolates. We showed that antigenically different strains of IBV are present in China. Some of these strains were highly pathogenic and H120 vaccine did not provide protection against challenge with them.

Material and Methods

Field strains

Specimens of trachea, kidney and cloacal swabs were collected from flocks with IB-like clinical disease. Specimens were homogenized with 4 vols of sterile PBS containing 1000 unit/ml of penicillin and 1 mg/ml of streptomycin. The homogenates were clarified by centrifugation at 3000 *g* for 30 min at 4°C, the supernatant collected and stored at -25°C. Before freezing, 0.2 ml of homogenate was inoculated into the allantoic sac of 9 to 11-day-old embryonated specific pathogen-free (SPF) eggs, five to seven eggs per specimen and eggs incubated at 37°C. From one egg of each specimen, allantoic fluid was collected at 48 h after inoculation and used as the inoculum for the next passage. The remaining eggs were incubated for 5 further days and examined for lesions specific for IBV. In total, each specimen received three egg passages before samples were discarded as negative or used for further characterization. The Tianjin strain was supplied by the Poultry Disease Diagnostic and Training Centre of the Municipality of Tianjin, whereas the Shenyang strain was supplied by the Veterinary College of Shenyang Agricultural University.

Chickens and eggs

SPF chickens and eggs were obtained from a SPF flock of White Leghorns maintained at the SPF facility of the Harbin Veterinary Research Institute.

Reference IBV strains and vaccines

H52 and H120 vaccines were supplied by a local vaccine manufacturer. Strains Massachusetts 41 (M41), Connecticut (Con), Gray, Arkansas 99 (Ark 99) and Australian T (T) were obtained from the former Houghton Laboratory, Houghton, Huntingdon, UK. Q1/76 strain is an Australian IBV strain described previously (Ignjatovic & McWaters, 1991). All strains were propagated in 9 to 11-day-old embryonated SPF eggs by inoculation of approximately 10³ median ciliostatic doses (CD₅₀) of virus into the allantoic sac. After incubation for 48 h at 37°C, allantoic fluid was collected, clarified by centrifugation at 3000 *g* and stored at -25°C. Virus titres were determined in tracheal organ cultures (TOC) as described below.

Antisera to IBV strains and serum neutralization (SN) test

Antisera were prepared by intra-ocular inoculation of approximately 10⁴ CD₅₀ of each IBV strain into a group of 10 SPF chickens at 3 and again at 7 weeks of age. Each group was housed in strict isolation in a negative pressure isolation unit. Sera were collected 4 weeks after the second inoculation, heat inactivated at 56°C and stored at -25°C until use. The SN test was performed in TOCs with 100 CD₅₀ of virus against log₂ dilutions of chicken sera (Darbyshire *et al.*, 1979).

Haemagglutination (HA) and haemagglutination inhibition (HI) tests

The HI test for IBV antibodies was conducted using 8 HA units of the M41 strain. The HA test for detection of the possible presence of

Newcastle disease virus (NDV) was carried out using the allantoic fluid from the third egg passage for each of the IBV strains.

TOC and infectivity assay

TOCs were prepared from SPF embryos at 20 days of incubation as previously described (Cook *et al.*, 1976). In brief, sections 1 mm in length were cut manually, placed into glass tubes with 1 ml of Dulbecco's modification of Eagle's medium and incubated in a rotary shaker at 37°C. Rings with 70 to 100% of cilia activity were selected 24 h later and inoculated with 0.1 ml of a test sample, in triplicate. The 100% loss of cilia activity at 5 days after inoculation in at least 2/3 rings was taken as indication of the presence of infectious virus in a test sample.

Cross-reaction with monoclonal antibodies in direct ELISA

The reaction of various IBV strains with monoclonal antibodies (Mabs) designated 1, 2, 5, 7, 16, 24, 27 and 51 directed against different epitopes on the nucleocapsid N (Mabs 1, 7, 16, 24, 27 and 51), membrane M (Mab 2) and peplomer S (Mab 5) proteins of IBV was determined using direct ELISA as previously described (Ignjatovic & McWaters, 1991). None of the Mabs, including Mab 5, are serotype specific. In brief, infected allantoic fluid was clarified at 3000 *g* and then centrifuged at 35,000 *g* for 1 h in a Sorvall high-speed centrifuge. Virus pellet was resuspended to 1/50 of its original volume in PBS and used after dilution, usually 1/10 or 1/20 in carbonate-bicarbonate buffer pH 9.6, to coat the wells of microtitre plates (Disposable Products, Adelaide, Australia). After overnight incubation at 37°C culture fluids of Mabs were added and after addition of goat anti-mouse IgG horseradish peroxidase conjugate (Kirkegaard & Perry Labs, Gaithersburg, Maryland, USA) and substrate, 5-aminosalicylic acid, absorbance at 450 nm was measured using an Titertek Multiscan reader (ICN Laboratories, Costa Mesa, CA, USA). Samples were considered positive if the mean absorbance at 450 nm exceeded 0.10 (signal to noise ratio greater than 2:1).

Challenge studies

SPF chicks, at one day of age were placed into 13 negative pressure isolation units, 15 chicks per unit in the animal facility of the Harbin Veterinary Research Institute, and given water and feed *ad libitum*. At one day of age chicks in groups 1, 3, 5, 7, 9 and 11 were vaccinated with H 120 vaccine by intra-ocular administration of the recommended dose in 0.1 ml. Chicks in groups 2, 4, 6, 8, 10, 12 and 13 were left unvaccinated. At 3 weeks of age, five chicks in each group, randomly selected, were bled from a wing vein, sera collected and stored at -25°C. At 3 weeks of age chicks in all groups, except for those in group 13, were challenged intra-ocularly with one of six field isolates of IBV, 10⁴ CD₅₀ in 0.1 ml of PBS. Five days after challenge, tracheal swabs were collected from all chicks, placed in 1 ml of sterile PBS containing 1000 unit/ml of penicillin and 1 mg/ml of streptomycin and stored at -70°C. Tracheal swabs were thawed and frozen three times and 0.2 ml of each swab, without any other treatment, assayed in TOCs, for the presence of infectious virus. Chicks in all experimental groups were examined daily for clinical signs of infection and deaths until 14 days after infection.

Clinical signs, post-mortem findings and mortality

Chicks were observed for signs of sneezing, gasping, depression, emaciation, reluctance to move, hunched posture with ruffled feathers, wet litter and soiled vent (Chong & Apostolov, 1982). For each experimental group, clinical signs were visually scored up to 14 days post inoculation. Respiratory noises such as rales and coughing were difficult to evaluate since chicks were kept within negative pressure isolation units. All dead birds were examined post-mortem for lesions suggestive of IBV infection, e.g. cloudiness, thickening and accumulation of cheesy exudate in the air sac membranes, inflammation and thickening of the membranes around the heart and liver, swollen, pale

Table 1. *Origin of IBV strains isolated*

Strain designation	Details of isolation				Clinical disease observed ^b
	Location	Date	Flock (age in days)	Vaccinated ^a	
YY	Yu Yao	1986	Pullets (90)	No	Nephritis
NB-90	Harbin	1990	Broilers (35)	No	Nephritis
HV	<i>Da Lian</i>	1991	Broilers (46)	No	Nephritis
NRZ	Shong Hua River	1991	Pullets (70)	No	Respiratory
H-93	Wang-Harbin	1993	Boilers (28)	Yes	Respiratory
TJ	Tianjin	1994	NK ^c	Yes	Nephritis
ShY-6	Shenyang	1995	Pullets (50)	No	Respiratory
AD (20)	An Da Heeling	1995	Pullets (28)	Yes	Nephritis

^aVaccinated with H120 or H52 vaccines at between 1 to 10 days and 3 to 4 weeks, respectively.

^bNephritis = swollen kidneys with deposits of urates in tubules; respiratory = tracheal rales, sneezing and gasping.

^cNK = not known.

Table 2. *Reaction of reference IBVs and field isolates with a panel of monoclonal antibodies in a direct ELISA*

IBV strains	Monoclonal antibody ^a								
	N							M	S1
	7	51	24	1	16	27			
Reference strains									
M41	- ^b	-	+	+	+	-	+	+	
H52	-	-	+	+	+	+	+	±	
H120	-	-	+	+	+	+	+	+	
Gray	-	-	+	+	+	+	+	+	
Con	-	-	+	-	-	-	+	-	
Ark99	-	-	+	-	-	+	+	+	
Q1/69	+	+	+	+	+	+	+	+	
Field isolated IBV strains									
HV	-	-	+	-	-	+	+	+	
NRZ	-	-	+	-	-	-	-	+	
NB-90	-	-	+	-	+	+	+	-	
TJ	-	-	+	-	-	+	+	+	
AD-20	-	-	+	-	-	+	-	-	
H-93	-	+	-	-	-	-	-	-	
YY	+	-	+	-	+	-	+	±	
SY-6	+	-	+	-	+	+	+	+	

^aMonoclonal antibodies directed against nucleocapsid (N), membrane (M) and S1 subunit of the peplomer.

^b+ or - = reaction or no reaction, respectively in direct ELISA.

or marbled kidneys, and deposits of urates in the ureters and cloaca (Winterfield & Hitchner, 1962; Chong & Apostolov, 1982).

Electron microscopy

For each putative IBV isolate, virus was pelleted from infected allantoic fluid at 35,000 g in a Sorvall high speed centrifuge, resuspended in PBS, negatively stained with 1% phosphotungstic acid and examined for the presence of virus particles with surface projections and size typical of coronaviruses.

Statistical analysis

The proportion of IB-positive and dead animals were tested with non-parametric Fisher's exact test, comprising vaccinated and non-

vaccinated groups that had been challenged with the same IBV strains. All groups were similarly compared to the control non-vaccinated, non-challenged group 13.

Results

Virus isolation and identification

Eight IBV isolates (Table 1) were recovered from samples collected between 1986 and 1995 on eight different farms located in four different provinces, Heilongjiang (NRZ, NB-90, H-93 and AD-20), Liaoning (HV and ShY-6), Tianjin (TJ) and Zhejiang (YY). Isolates were either from broilers or

Table 3. Cross-neutralization titres between four IBV strains isolated in China and reference strains

IBV strain	Titre with antisera to IBV strain ^a							
	HV	NB90	NRZ	TJ	M41 ^b	Gray ^b	T ^b	Con ^b
HV	512 ^c	128	8	64	8	8	32	8
NB-90	64	256	8	32	8	256	128	16
NRZ	4	2	128	2	64	2	2	8
TJ	32	4	16	32	4	4	4	2
M41	4	8	64	8	128	8	16	32
Gray	16	256	2	8	8	128	256	32
T	16	64	8	32	4	64	128	16
Con	8	16	16	2	32	32	32	256

^aReciprocal of the highest dilution of sera which neutralised 100 median ciliostatic doses of virus in tracheal organ cultures.

^bReference strains.

^cHomologous titres in bold.

Table 4. Protection by H120 vaccine against challenge with IBV field isolates

Group ^a	Vaccine used ^b	Challenge strain ^c	No positive for virus/ no tested (%) ^d	No dead/15 inoculated (%) ^e
1	Vaccinated	HV	6/15 (40) ^{A,D}	4 (27) ^{A,H}
2	NV ^f	HV	7/15 (47) ^{A,D}	5 (33) ^{A,H}
3	Vaccinated	NB-90	6/15 (40) ^{A,E}	6 (40) ^{A,I}
4	NV	NB-90	9/15 (60) ^{A,E}	9 (60) ^{A,I}
5	Vaccinated	NRZ	0/15 (0) ^B	0 (0) ^J
6	NV	NRZ	4/15 (27) ^{A,B}	1 (7) ^J
7	Vaccinated	YY	7/15 (47) ^{A,F}	5 (33) ^{A,K}
8	NV	YY	9/15 (60) ^{A,F}	7 (47) ^{A,K}
9	Vaccinated	ShY-6	0/15 (0) ^C	0 (0) ^L
10	NV	ShY-6	7/15 (47) ^{A,C}	2 (13) ^L
11	Vaccinated	TJ	11/12 (92) ^{A,G}	13 (81) ^{A,M}
12	NV	TJ	10/13 (77) ^{A,G}	14 (90) ^{A,M}
13	—	None	0/15 (0)	0 (0)

^aFifteen SPF chicks, housed separately in positive pressure isolators.

^bCommercial vaccine given by ocular route at the recommended dose to day-old chicks.

^cApproximately 10⁴ median ciliostatic doses of each strain, in 0.1 ml, given at 3 weeks of age by ocular route.

^dPresence of infectious virus in tracheal swabs at day 5 after challenge.

^eCumulative mortality at 14 days after challenge.

^fNV = not vaccinated.

A = significantly different ($P \leq 0.05$) in pair-wise comparison to the control group 13; B and C significantly different ($P \leq 0.05$) from each other; D–M = values with the same superscript not significantly different ($P \geq 0.05$) from each other in pair-wise comparison.

young layers, of which the majority (5/8) were not vaccinated. Clinical disease described as nephritis with mortality was more frequently observed than was respiratory disease and occurred in both vaccinated and non-vaccinated flocks. By electron microscopy, all eight isolates had typical coronavirus morphology and were free of other agents such as NDV (results not shown). They were also free from NDV by HA test. All eight isolates at the third egg passage level induced lesions typical of IBV in embryonated eggs.

Antigenic characterization of strains

The eight IBV isolates and seven reference strains were compared for their reaction with eight Mabs (Table 2). The M41, H52, H120 and Gray strains reacted similarly with the eight Mabs, whereas Con and Ark 99 strains differed. All eight IBV isolates differed from H52 or H120 in their crossreaction with the panel of Mabs. Strains NB-90 and ShY-6 were the most similar to H120, whereas strains NRZ, AD-20 and H-93 were the most different.

Serotyping was also performed with four strains HV, NB-90, NRZ and TJ and reference strains in TOCs (Table 3). NRZ was found to be of the Massachusetts serotype whereas strains HV, NB-90 and TJ were not. HV and NB-90 showed serological relatedness to each other. TJ strain was also related to HV and NB-90 strains and also in a one-way reaction to the T strain. NB-90 was similar to Gray and T strains, which also shared antigenic similarity with each other.

Protection induced by H120 vaccine

The level of protection to challenge with each of six isolates was evaluated in chicks which had been vaccinated with H120 at one day of age and challenged three weeks later (Table 4).

Three weeks after vaccination all vaccinated chicks had antibodies to IBV indicative of an effective vaccination response. Reciprocal HI titres were in the range of 32 to 512, with the exception of one chick in group 9. Chicks in the non-inoculated groups were HI negative, except for one chick in group 12 which had an HI titre of 1:64.

Following challenge, virus was detected in the tracheal swabs from all non-vaccinated and challenged groups. The proportion of virus positive swabs in all groups were significantly different ($P \geq 0.05$) from the control group 13 (Table 4). Mortality occurred in all non-vaccinated challenged groups. Cumulative mortality at 14 days after challenge with four strains HV, NB-90, YY and TJ strains was 33, 60, 47 and 90%, respectively, and these were significantly different from the control group 13, in which no mortality occurred. In the groups challenged with NRZ and ShY-6 strains, cumulative mortality was not significantly different from the control group.

H120-vaccinated chicks were protected against challenge with two strains, NRZ and ShY-6 as determined by the absence of virus recovery 5 days after challenge. In the corresponding non-vaccinated groups only 4/15 and 7/15 swabs, respectively, were positive for virus. However, the difference between the number of virus positive swabs in the corresponding vaccinated and non-vaccinated groups was statistically significant ($P \leq 0.05$). No mortality occurred in vaccinated groups challenged with NRZ and ShY-6, in comparison to 7 and 13% mortality in non-vaccinated groups. However, these differences were not statistically significant ($P \geq 0.05$).

Chicks vaccinated with H120 and challenged with HV, NB-90, YY and TJ strains were not protected as determined by both virus isolation and mortality and in pair-wise comparison were not significantly different. Mortality rate in the vaccinated groups challenged with HV, NB-90, YY and TJ were all reduced in comparison to the non-vaccinated groups, but not significantly.

In a further experiment in which chicks were

vaccinated with H120 vaccine at 3 weeks of age, and challenged with HV, NB-90 and NRZ strains 8 days later, similar results were obtained to those reported here. H120 vaccinated chicks were protected against challenge with NRZ but not against challenge with HV or NB 90 (results not shown).

Assessment of pathogenicity of field IBV strains

In non-vaccinated groups which were infected with HV, NB-90, YY and TJ at 3 weeks of age, the first clinical signs appeared after 4 days; the majority of chicks showed depression, but no apparent respiratory disease. Mortality began at 5 days after challenge and ceased by day 10 in all groups except group 12, inoculated with TJ strain, where 14/15 chicks died by day 7 after infection. In the H120-vaccinated groups challenged with HV, NB-90, and YY, the first clinical signs also appeared at 4 days after challenge, however, onset of mortality was delayed in comparison to the non-vaccinated groups until day 7 and ceased by day 10. The exception was group 12, in which there was no delay in onset of either clinical signs or mortality. In the non-vaccinated groups challenged with HV, NB-90, YY or TJ strains the majority of chickens that died had swollen kidneys, some of which were marbled. The kidneys and urogenital tracts of some chicks contained deposits of uric acid. In the H120-vaccinated groups challenged with either NRZ or ShY there were no apparent clinical signs.

Discussion

The IBV strains reported here were isolated from broiler and pullet flocks located in different regions of China, confirming that IBV might have contributed to mortality and loss in production in China for a considerable period.

Antigenic characterization of the eight IBVs showed that the majority differed antigenically from both M41 and H120. Six strains, NRZ, NB-90, AD-20, H-93, YY and ShY-6, differed from each other and from M41 and H120 by Mab analysis, whereas two strains, HV and TJ, were similar to each other but differed from M41 and H120. Antigenic differences detected by Mabs were in some instances relatively small; i.e. strains lacked one or two epitopes on the N protein in comparison to H120. It should be considered, however, that the Mabs used in this study had been developed against Australian IBV strains. Although antigenic relationship between Australian and IBV strains from other countries has not been determined, previous comparisons at the nucleotide level between the USA strains used in this study and Australian strains has indicated that they differ considerably and belong to a separate genetic lineages (Sapats *et al.*, 1996a,b). Antigenic differentiation of field and reference IBV strains by serum neutralization was less clear due to a degree of

cross-reaction. From four strains serotyped, NRZ was similar to the Massachusetts serotype whereas HV, NB-90 and TJ were antigenically related, and differed from both Massachusetts and Connecticut serotypes. Serologically, HV, NB-90 and TJ were not identical: TJ showed only one-way antigenic similarity with HV and NB-90; NB-90 was similar to nephropathogenic Gray and T strains, whereas HV was not; TJ showed antigenic similarity to the T strain, again only in a one-way reaction. Recently, Wang *et al.*, (1997) reported the isolation of an IBV strain from the Sichuan province, which was also serologically related to the Australian T strain. It must be noted however that in this study T and Gray strains were antigenically related, which is in contradiction to the results of previous studies (Darbyshire *et al.*, 1979; Wang *et al.*, 1997). The reason of this discrepancy is not clear.

A further finding from the present study was that from six strains tested for pathogenicity, four were highly pathogenic and able to induce significant mortality. The clinical signs, as well as gross pathology induced by all four strains HV, NB-90, YY and TJ were identical to those attributable to the nephropathogenic T strain and also other nephropathogenic strains (Winterfield & Hitchner, 1962; Cumming, 1963; Chong & Apostolov, 1982).

The isolation of nephropathogenic strains from flocks vaccinated with Massachusetts type vaccine have been reported in other countries (Meulemans *et al.*, 1987; Zanella, 1988; Kinde *et al.*, 1991; Lin *et al.*, 1991; Picault *et al.*, 1991; Lambrechts *et al.*, 1993). Their prevalence in many countries, excluding Australia, however still remains relatively low, although in France nephropathogenic strains might be currently the most prevalent strains (Picault *et al.*, 1991). Isolation of at least four nephropathogenic strains in China that are capable of causing high mortality in SPF chicks has thus confirmed the clinical observation of disease in the field. Furthermore their isolation from at least four provinces lends support for the view that the prevalence of nephropathogenic IBV strains is likely to be high in many parts of China. Zhang *et al.* (1995) and Wang *et al.* (1997) also recently reported isolation of a nephropathogenic strains from Tianjin, Huhhot and Sichuan which caused 60 to 90% mortality. While the reason for the appearance of nephropathogenic IBV strains still remains somewhat speculative, the likelihood of a linkage with the high protein diet in feed should not be discounted (Cumming & Chubb, 1988).

Importantly, H120 vaccines did not protect against challenge by four out of six IBV strains isolated in this study. All four strains were highly pathogenic, capable of causing between 33 to 90% mortality from nephritis. Immunity induced by H120 vaccine failed to prevent virus replica-

tion in the trachea or mortality. In this study, the isolation rate of challenge virus from most non-vaccinated groups was lower than expected, although all of these values were significantly different from the control group. Challenge virus was assayed in tracheal swabs and the method of swab collection or subsequent treatment might have contributed to the lower virus isolation rate.

Lack of protection by H120 vaccines against challenge with nephropathogenic strains was demonstrated previously and necessitated introduction of vaccines to control these strains (Zanella, 1988; Lambrechts *et al.*, 1993; Pensaert & Lambrechts, 1994). The likelihood that bivalent IB vaccines, incorporating both Massachusetts and nephropathogenic strains may be needed to control IBV in China is therefore strong (Ding & Wu, 1994). It should be noted that in the present study we have not determined whether cross-protection exists between these four nephropathogenic isolates.

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RÉSUMÉ

Caractérisation antigénique et immunogène des souches du virus de la bronchite infectieuse isolées en Chine entre 1986 et 1995

Huit souches de virus de la bronchite infectieuse (IBV) ont été isolées entre 1986 et 1995 à partir de poulets de chair et de poudeuses dans huit fermes différentes de quatre provinces chinoises. Les virus ont été isolés à partir de troupeaux qui présentaient soit des symptômes respiratoires, soit de la néphrite, et dont la majorité n'avait pas été vaccinée contre l'IBV. Les anticorps monoclonaux ont permis de différencier six souches entre-elles et également des souches H120, Connecticut et Arkansas 99. Quatre de ces souches ont été sérotypées: une (NRZ), appartenait au sérotype Massachusetts, trois (HV, NB-90 et TJ) possédaient une antigénicité commune et appartenaient à un sérotype différent des souches Massachusetts et Connecticut. La souche NB-90 était similaire aux souches Gray et T, alors que la souche TJ possédait quelque similarité avec la souche T. Quatre souches, HV, NB-90, YY et TJ, ont induit respectivement 33, 47, 60 et 90% de mortalité chez des poulets SPF âgés de trois semaines. Les signes cliniques et les lésions post mortem ont été identiques à ceux induits par la souche néphropathogène T. Les poulets vaccinés avec la souche H120 puis éprouvés avec les quatre souches très pathogènes HV, NB-90, YY et TJ, n'ont pas été protégés comme l'ont révélé le réisolement du virus et la mortalité. Les résultats montrent que l'on trouve fréquemment dans les troupeaux de volailles en Chine, des souches d'IBV très pathogènes qui induisent des néphrites cliniques. Ils confirment aussi les observations terrain en ce qui concerne l'absence de protection conférée par les vaccins couramment utilisés de la bronchite infectieuse de sérotype Massachusetts vis-à-vis de ces souches néphropathogènes.

ZUSAMMENFASSUNG

Antigene und immunogene Charakterisierung von Bronchitisvirus-Stämmen, die zwischen 1986 und 1995 in China isoliert wurden

Acht Bronchitisvirus (IBV)-Stämme wurden zwischen 1986 und 1995 aus Broilern und Legehühnern in acht verschiedenen Farmen in vier Provinzen in China isoliert. Die Viren wurden aus Herden isoliert, die entweder unter Respirationskrankheiten oder unter Nephritis litten und größtenteils nicht gegen IBV geimpft worden waren. Bei sechs Stämmen wurde mit Hilfe von monoklonalen Antikörpern nachgewiesen, daß sie sich von den IBV-Stämmen H120, Connecticut und Arkansas99 sowie auch untereinander unterschieden. Vier dieser Stämme wurden serotypisiert; einer (NRZ) war vom Serotyp Massachusetts, drei (HV, NB-90 und TJ) teilten eine gewisse Antigenähnlichkeit und gehörten einem Serotyp an, der sich von Massachusetts und Connecticut unterschied. NB-90 war den Stämmen Gray und T ähnlich, während TJ eine gewisse Ähnlichkeit mit dem Stamm T hatte. Vier Stämme, und zwar HV, NB-90, YY und TJ, verursachten bei drei Wochen alten spezifisch-pathogenfreien Küken eine Mortalität von 33%, 47%, 60% bzw. 90%. Die klinischen Symptome und die Sektionsbefunde waren mit denen identisch, die durch den nephropathogenen Stamm T verursacht wurden. Küken, die mit dem Stamm H120 vakziniert und danach mit den vier stark pathogenen Stämmen HV, NB-90, YY und TJ testinfiziert wurden, waren nicht geschützt, was sowohl durch Virusisolierungen als auch durch die Mortalität festgestellt wurde. Die Ergebnisse zeigen, daß stark pathogene IBV-Stämme, die eine klinische Nephritis verursachen, in Geflügelbeständen in China häufig vorkommen. Sie bestätigen auch Praxisbeobachtungen über den mangelnden Impfschutz durch die zur Zeit verwendeten IB-Vakzinen vom Serotyp Massachusetts gegen die Infektion mit diesen nephropathogenen Stämmen.

RESUMEN

Caracterización antigenica e inmunogenica de cepas del virus de la bronquitis infecciosa, aisladas en China, entre 1986 y 1995

Se aislaron ocho cepas del virus de la bronquitis infecciosa (IBV) a partir de broilers y ponedoras en ocho granjas diferentes de cuatro

provincias de China. Los virus se aislaron de explotaciones en las que los animales presentaban un cuadro respiratorio o nefritis, y la mayoría no habían sido vacunados frente a IBD. Mediante anticuerpos monoclonales se evidenciaron seis cepas que no correspondían a las cepas H120, Connecticut y Arkansas 99 y que eran diferentes entre sí. Cuatro de estas cepas se serotiparon, una (NRZ) era del serotipo Massachusetts, mientras que las otras tres (HV, NB-90 y TJ) que presentaban un grado de antigenicidad similar, pertenecían a un serotipo diferente del Massachusetts y Connecticut. La cepa NB-90 presentaba semejanzas con las cepas Gray y T mientras que la TJ compartía alguna semejanza con la cepa T. Cuatro cepas HV, NB-90, YY y TJ daban lugar a un 33, 47, 60 y 90% de mortalidad,

respectivamente, en pollos SPF de tres semanas de vida. El cuadro clínico y los hallazgos post mortem observados, eran idénticos a los producidos por la cepa T nefrotóxica. Los pollos vacunados con la cepa H120 e infectados con cuatro cepas patógenas (HV, NB-90, YY, y TJ) no quedaban protegidos, como demostró la elevada mortalidad y el aislamiento de los virus respectivos. Los resultados muestran que en explotaciones aviares de China, se dan frecuentemente infecciones por cepas de IBD altamente patógenas que producen cuadros clínicos de nefritis. También confirmaron las observaciones de campo acerca de la falta de protección de las vacunas de IBD del serotipo Massachusetts, normalmente utilizadas contra estas cepas nefrotóxicas.