SEROPREVALENCE AND POLYPEPTIDE ANALYSIS OF INFECTIOUS BRONCHITIS VIRUS IN BROILERS

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ABSTRACT

A total of 360 trachea, kidneys and serum samples were collected from 21 broiler poultry farms having no history of vaccination against infectious bronchitis (IB). The agar gel precipitation and trypsin induced haemagglutination inhibition tests revealed 2.22% seroprevalence of IB. Data analysis revealed significantly ($P \leq 0.031$) higher IB seroprevalence in 1-2 weeks old broilers (5.36%), while the lowest prevalence was found in the older birds. After purification, IB virus was analyzed for polypeptides applying SDS-PAGE. The indigenous IB virus consisted of six major polypeptides with molecular weights of 84, 51, 36, 31, 28 and 23 kDa.

Key words: Infectious bronchitis virus, seroprevalence, haemagglutination inhibition test, polypeptide profile.

INTRODUCTION

Infectious bronchitis (IB) is an economically important disease of poultry and has worldwide distribution, with a morbidity and mortality up to 80 and 20%, respectively (Gelb *et al.,* 1991). Although trachea is the primary predilection site, the virus also multiplies in kidneys, intestines and oviducts.

The unique amino acid sequence, epitopes and glycoproteins determine the serotype of a virus. The IB virus has been reported to contain up to six major proteins i.e. four glycoproteins of molecular weights 84, 36, 31 and 28 kDa and two non-glycosylated proteins of molecular weights of 51 and 23 kDa (Stern *et al.,* 1982). In Pakistan, no attempt has been made to study the protein profile of indigenous IB virus. The present study was, therefore, executed to know the seroprevalence of IB in broilers and protein profile of the indigenous IB virus isolated from these birds.

MATERIALS AND METHODS

Trachea, kidneys and serum samples were collected from 360 birds belonging to 21 poultry farms in the areas of Faisalabad, Jhang and Kamalia, where birds were not vaccinated against infectious bronchitis (IB). These birds were divided into three age groups i.e., 1-2 (n = 112), 3-4 (n = 123) and 5 (n = 125) weeks of age. Trachea and kidney samples were stored in sterile polythene bags having transport medium composed of Penicillin (10,000 IU) and Streptomycin (10 mg/mL) (Gelb *et al.,* 1991). Tissue and serum samples were stored at -20^{o} C till further use.

The prevalence of IB was evaluated by performing agar gel precipitation test (AGPT) and trypsin-induced haemagglutination inhibition (HI) test, following the method of Lashgari and Newman (1984). For the AGPT, lyophilized IB vaccine virus (Massachusetts M-41) was reconstituted to serve as antigen.

The samples positive for the IB virus were sedimented through 6.5 mL of 20% sucrose in the TNE (50 mM tris HCL, 50mMNaCl, 1mM EDTA) on to a cushion of 5 mL of 55% sucrose. Centrifugation was carried out in a SW-27 rotor at 75000 x g for 3 hours. The samples were located, aspirated and diluted in TNE and centrifuged to equilibrium in 16 mL linear 20 to 55% sucrose TNE gradients at 75000 x g for 6 hours in an SW-27 rotor. Finally, the samples were diluted with TNE and pelleted by sedimentation at 65000 x g for 3 hours. The pellets were stored at –65 °C until use.

Purified virus was suspended in sample buffer, boiled for 1 minute and analyzed through SDS-PAGE electrophoresis on discontinuous 12.5% separating gel and 3.5% stacking gel (Laemmli, 1970). A 20 uL amount of sample was loaded in each well. A standard broad range recombinant protein marker (Bench Mark, 6-225 kDa) was used for comparison. Electrophoresis was performed at 175v for 2 hours at room temperature. The gels were stained with Coomasie brilliant blue for over night and then de-stained with methanol and glacial acetic acid for 6 hours. Chi-square test was applied to know the significant difference among various age groups.

RESULTS AND DISCUSSION

Infectious bronchitis (IB) is an acute, highly contagious viral respiratory disease of chickens characterized by tracheal rales, coughing and sneezing

(Gelb *et al.,* 1991). Birds of all ages are susceptible to IB virus but the disease is most severe in baby chicks causing mortality (Hofstad, 1984).

In the present study, seroprevalence of the IB virus was found to be 2.22% (Table 1). Similar incidence (2.63%) has been reported by Ahmad *et al.* (1986).

Several IB virus specific proteins have been identified, out of which three are most important, the spike (S) glycoprotien, the membrane (M) glycoprotein and the nucleocapsid (N) protein (Lai and Cavanagh, 1997). In addition, a fourth protein (small membrane protein, E) is believed to be associated with the virion

Table 1: Seroprevalence of infectious bronchitis in Faisalabad, Jhang and Kamalia, Pakistan

Localities	Age (weeks)	Samples tested (No.)	Positive samples		Affected birds (%)
			AGPT	HA/HI	
Faisalabad	1-2	43	3	3	6.98
(n=127)	3-4	44	0	0	0.00
	5	40	0	0	0.00
Jhang (n=124)	1-2	37	2	2	5.41
	3-4	43	0	0	0.00
	5	44	1	1	2.27
Kamalia (n=109)	1-2	32	1	1	3.13
	3-4	36	1	1	2.78
	5	41	0	0	0.00
Overall		360	8	8	2.22

AGPT = Agar gel precipitation test; HA = Haemagglutination; HI = Haemagglutination inhibition.

The data analysis revealed that seroprevalence of the IB was significantly ($\chi 2 = 6.920$; $P \leq 0.031$; df = 2) higher in birds of 1-2 weeks (5.36%), while the lowest prevalence was found in 3-4 and 5 weeks old birds (Fig. 1). As age increases, chicken become more resistant to the IB virus and mortality becomes less (Smith *et al.,* 1985; Albassam *et al.,* 1986).

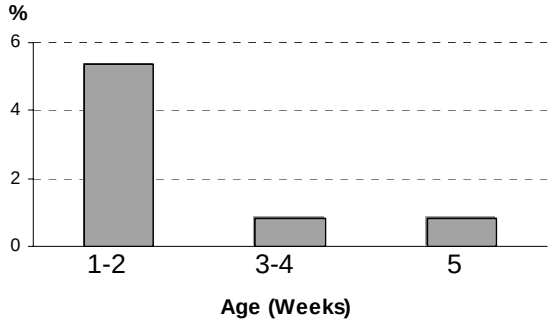

Fig. 1: Age-based seroprevalence of IB virus in broilers based on AGPT and HA/HI tests.

The trypsin induced haemagglutination inhibition and agar gel precipitation tests were found to be suitable sero-diagnostic techniques for the detection of antibodies against the IB. In trypsin induced HA and HI tests, the results remained stable for 2 hours and trypsin at a concentration of 2% was found very specific to elicit the haemagglutinating activity in the IB (Mahmood *et al*., 2004). The agar gel precipitaion test proved to be very efficient in detecting the presence of precipitating antibodies, which appeared 7 days after infection and persisted up to 94 days post infection (Witter, 1962).

envelope in very small amounts and is essential for virus particle formation. The S protein comprises two or three copies of each of two glycopolypeptides, S1 and S2 (approximately 520 and 625 amino acids, respectively). Haemagglutination-inhibiting and most of the virus-neutralizing antibodies are induced by S1 (Jackwood *et al*., 1992; Ignjatovic *et al.,* 1997). Only about 10% of the M protein is exposed at the outer virus surface. The N protein is around the single piece of single-stranded, positive sense, RNA genome that comprises approximately 27,600 nucleotides, the whole of which has already been cloned and sequenced (Bäyon-Auboyer *et al.,* 1999). Preparations of purified virus always contain host cell polypeptides. The S2 protein can be difficult to detect by Coomassie blue staining and some N protein can be missing or degraded.

In the present study, the SDS-PAGE revealed that the IB virus was made up of six proteins with molecular weights of 84, 51, 36, 31, 28 and 23 kDa (Fig. 2). Stern *et al.* (1982) also reported a similar polypeptide profile of IB virus when subjected to SDS–PAGE. Differences in the polypeptide profile can be attributed to different disruption or purification techniques (Yu *et al.,* 2001). The present study has indicated that the indigenous IB virus has six proteins. With this in sight, it is imperative that those strains which resemble closely with the indigenous virus should be used for vaccine production.

Since the present study has indicated the prevalence of IB in broilers, suitable biosecurity measures must be taken to tackle the problem. The polypeptide profile has revealed that the IB virus contains six proteins, which closely resembles the polypeptide profile of the M-41 strain of IB. Further

studies are suggested to establish a correlation between the local IB virus strains and the vaccinal M-41 IB virus strain.

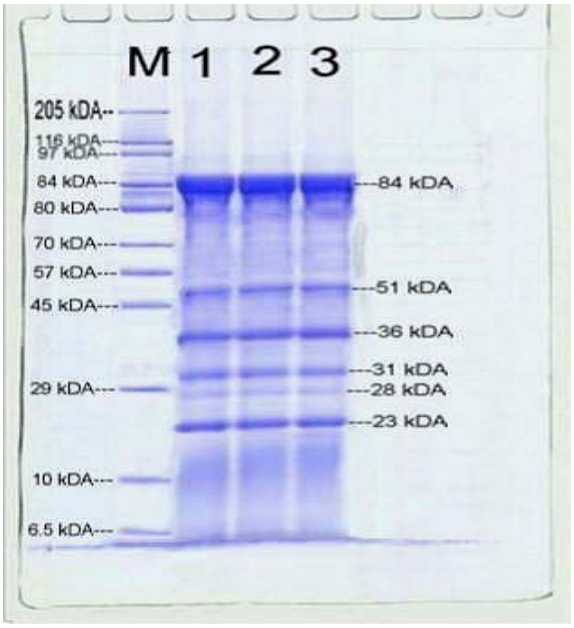

Fig. 2: The polypeptide profile of IB isolates by SDS-PAGE from three locations (lane 1 = Isolate from Jhang; lane 2 = Isolate from Kamalia; lane 3 Isolate from Faisalabad). Each isolate is comprised six polypeptides of 84, 51, 36, 31, 28 and 23 kDa. M = Protein marker (Bench Mark protein ladder, Sigma Aldrich).

REFERENCES

Ahmad, J., M. Ashfaque, M. Irfan and Anwar–ul-Haq, 1986. Incidence of the infectious bronchitis in poultry. Pakistan Vet. J., 6: 19-20.

Albassam, M. A, R. W. Winterfield and H. L. Thacker, 1986. Comparison of the nephropathogenicity of four strains of infectious bronchitis virus. Avian Dis., 30: 468-476.

Bäyon-Auboyer, M. H., V. Jestin, D. Toquin, M. Cherbonnel and N. Eterradossi, 1999. Comparison of F-, G- and N-based RT-PCR protocols with conventional virological procedures for the detection and typing of turkey rhinotracheitis virus. Arch Virol., 144: 1091-1109.

Gelb, J., J. B. Wolff and C. A. Moran, 1991. Variant serotypes of IBV isolated from the commercial layer and broiler chicken. Avian Dis., 35: 82-87.

Hofstad, M. S., 1984. Avian infectious bronchitis. In: Hofstad, M.S., H. J. Barnes, B. W. Calnek and W. M. Reid, Jr. (Eds). Diseases of Poultry. 8th Ed., Iowa State Univ. Press, Ames, Iowa, USA, pp: 429-443.

Ignjatovic, J., S. I. Sapats, and F. A. Ashton, 1997. Long term study of Australian infectious bronchitis viruses indicates a major antigenic change in recently isolated strains. Avian Pathol., 26: 535-552.

Jackwood, M. W., H. M. Kwon and D. A. Hilt, 1992. Infectious bronchitis virus detection in allantoic fluid using the polymerase chain reaction and a DNA probe. Avian Dis., 36: 403-409.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature, 227: 173-185.

Lai, M. M. C. and D. Cavanagh, 1997. The molecular biology of coronaviruses. Advances in Virus Res., 48: 1-100.

Lashgari, M. S. and J. A. Newman, 1984. Serological comparison and antigenic relationships of seven serotypes of infectious bronchitis virus using the haemagglutination-inhibition test. Avian Dis., 28: 435-443.

Mahmood, M. S., M. Siddique, I. Hussain and A. Khan, 2004. Trypsin-induced haemagglutination assay for the detection of infectious bronchitis virus. Pakistan Vet. J., 24: 54-57.

Smith, H. W., J. A. K. Cook and Z. E. Parsell, 1985. The experimental infection of ckickens with mixtures of infectious bronchitis virus and *E. coli*. J. Gen. Virol., 66: 777-786.

Stern, D. F., L. Burgess and B. M. Sefton, 1982. Structural analysis of virion proteins of the avain coronavirus infectious bronchitis virus. J. Virol., 42: 208-219.

Witter, R. L., 1962. The diagnosis of infectious bronchitis of chicken by agar gel precipitation test. Avian Dis., 6: 478-492.

Yu, L., Y. Jiang, S. Low, Z. Wang, S. J. Nam, W. Liu and J. Kwangac, 2001. Characterization of three infectious bronchitis virus isolates from China associated with proventriculus in vaccinated chickens. Avian Dis., 45: 416-424.