

ELECTROPHORETIC PATTERN OF POLYPEPTIDES OF INFECTIOUS BURSAL DISEASE VACCINE VIRUS

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ABSTRACT

Four live vaccines and five field isolates of infectious bursal disease virus (IBDV) were analyzed for polypeptide profile using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Field viruses were purified and concentrated through sucrose gradient centrifugation. All the five field IBDV contained five polypeptides with molecular weights (MW) 27.70, 32.70, 41.60, 63.09 and 91.20 KDa. Out of four vaccinal strains of IBDV, only one vaccinal strain contained five polypeptides with almost similar MW i.e., 26.0, 32.0, 40.8, 62.0 and 90.9 KDa and one extra band (MW 20.3 KDa). One vaccine virus had three polypeptides with MW 40.8, 62.0 and 90.9 KDa and the remaining two vaccines contained two polypeptides with MW 40.8 and 90.9 KDa. Results reveal that vaccinal strains of IBDV differ from local field isolates in the sense of low protein profile or missing of some polypeptides.

INTRODUCTION

Infectious bursal disease (IBD), also called as Gumboro disease is one of the major threat to the poultry industry in Pakistan, by inflicting insidious economic losses (Anjum *et al.*, 1993). It is an acute, highly contagious viral infection of young chickens that targets primarily lymphoid tissue with a special predilection for the bursa of Fabricius (Dobos, 1979). IBDV is a member of family Birnaviridae including viruses with a genome consisting of two segments of double stranded RNA (Dobos *et al.*, 1979). The IBDV has four viral proteins named as VP1, VP2, VP3 and VP4 and a precursor protein VPX with respective molecular weights of 28, 32, 40, 47 and 91 KDa. The major host protective immunogenic protein is VP2 that elicits neutralizing antibodies known for serotype and strain specificity (Becht *et al.*, 1988).

IBDV exists worldwide in at least two antigenically distinct serotypes I and II and only serotype I, which displays a wide variation in pathogenic potential, is virulent for chickens. Recently, antigenically different variant strains have been recognized as a common cause of the failure in vaccination programs (Jackwood and Saif, 1987).

Prophylactic and control measures against Gumboro disease using variety of whole virus vaccines are in practice but complete success has not been achieved. One possible reason for this failure may be the variation among the strains of IBDV, due to variable cleavage of the precursor proteins (Jackwood *et al.*, 1984). The

present studies were, therefore, designed to illustrate the antigenic variations of different field and vaccinal IBDV strains using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The information thus procured would help further studies on cross protection trials through natural and experimental challenges.

MATERIALS AND METHODS

Vaccines

Four live infectious bursal disease virus (IBDV) vaccines purchased from the local market were

1. Bursine-2 (Solvay, USA)
2. Bio Gumboro (Bioteke, Italy)
3. Gumboro vaccine Nobilis Strain D-78 (Intervet, Holland)
4. Gumboral CT (Rhone Poulenc, France)

Field virus

Infected bursae of Fabricius from five different field outbreaks of infectious bursal disease (IBD) in and around Faisalabad were collected in sterile polythene bags and preserved at -20°C. Infected bursae were homogenized in phosphate buffered saline (PBS), centrifuged at 1200g for 20 minutes and the supernate was collected.

The supernate was mixed with chloroform at 1:1 and recentrifuged at 1200g for 20 minutes to remove tissue debris. Half of the clear supernate was sonicated in a jacketed vessel using Rapidis 600 (Ultrasonic Ltd.,

Germany) generating ultrasonics of an intensity of 75 watts per cm². Two strokes of 30 seconds each, were given with an intermission of 10 seconds to inactivate IBDV (Schneider and Haas, 1969).

Confirmation of the virus

The presence of IBDV in supernates of suspect homogenates was established through serological tests like indirect haemagglutination (IHA) and agar gel precipitation tests (AGPT). The hyperimmune serum was raised against a live IBD vaccine (D-78, Intervet, Holland) in rabbits as performed by Barnes *et al.* (1982). The antiserum was inactivated at 56 °C for 30 minutes in a water bath and stored at -10 °C for further use. The IHA test was conducted following the method of Rehman *et al.* (1994) whereas for AGPT the procedure mentioned by Asi and Iyisan (1991) was followed.

Concentration of field IBDV

Sucrose gradient centrifugation as described by Nagy and Lominiczi (1984) was adopted for the concentration and partial purification of field IBDV. The pellet obtained from unsonicated viral suspension was stored at -70 °C after adding 1% sodium dodecyl sulphate (SDS). Regarding pellet obtained from the sonicated viral fluid, the sonication and centrifugation procedures, mentioned earlier, were repeated and the pellet was stored at -70 °C for further use.

Concentration and purification of vaccine virus

Each vaccine was reconstituted in 5 ml of PBS solution and was processed as described for field isolates.

Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE with discontinuous buffer system in vertical fashion described by Laemmli (1970) was adopted for the segregation of polypeptides of IBDV. Separating gel (12.5%) and stacking gel (3.5%) were used (Maniatis *et al.*, 1982). Six protein markers as lysozyme, bovine serum albumin (BSA) dimer, BSA monomer, chicken egg albumin, carbonic anhydrase and haemoglobin of known molecular weights were used. The sample for loading was prepared by using 1X sample buffer instead of 2X sample buffer. Amount of sample which was loaded in wells of the casted gel was 30 µl. Electrophoresis was performed at room temperature using a constant current supply of 100 volts for 8 hours. The process was stopped when bromophenol blue dye was 1 cm above the bottom of the separating gel.

Gels were stained with Coomassie brilliant blue stain for over night and destained with a solution of methanol and glacial acetic acid for 6 hour with agitation. Molecular weights of the polypeptides of IBDV were calculated from a standard curve drawn between Rf values and log molecular weights of protein markers.

RESULTS

Polypeptide profile of infectious bursal disease virus (IBDV) field isolates and that of commercial vaccinal strains were compared through sodium dodecyl sulphate- polyacrylamide gel electrophoretogram studies.

Identification of field IBDV

The presence of IBDV in the field isolates and vaccines was checked through indirect haemagglutination (IHA) and agar gel precipitation tests. Geometric mean titres in IHA test of both vaccine virus and field isolates are presented in Table 1. Gel precipitation reactions for known positive control and field samples positive for IBDV produced visible precipitin line between central antiserum well and the surrounding sample wells.

SDS-PAGE comparisons

SDS-PAGE analysis of unsonicated field IBDV isolates along with marker proteins are shown in plate 1. The molecular weights (MW) of protein markers and their Rf values are presented in Table 2. No peptide band was observed for unsonicated field sample run.

Sonication

SDS-PAGE analysis of sonicated field IBDV isolates are presented in plate 2. All the field isolates of IBDV yielded five polypeptides when pellets were subjected to SDS-PAGE. In all the five lanes of sonicated field isolates, polypeptides showed almost similar Rf values as 0.596, 0.543, 0.460, 0.216 and 0.198. Their respective approximate MW were 27.70, 32.70, 41.60, 63.09 and 91.20 KDa. All the field viruses showed similar pattern of polypeptide segregation.

Vaccinal IBDV

The results of vaccinal IBD viruses purified through the sucrose gradient centrifugation are presented in plate 3. Using SDS-PAGE with 12.5% gel, four vaccinal strains of IBDV showed variable pattern of

Table 1: Identification of field IBDV isolates and commercial vaccinal strains by Indirect Haemagglutination test.

Outbreak	GMT	Vaccines	GMT
A	64	A	512
B	32	B	356
C	256	C	256
D	512	D	256
E	128		

Table 2: Rf values and log molecular weights of protein markers

Protein markers	Rf values	Mol Wts. (KDa)	Log Mol (Wts.)
Bovine serum albumin (BSA) Dimer	0.147	132	5.121
BSA monomer	0.320	66	4.819
Chicken egg albumin (CEA)	0.440	45	4.653
Carbonic anhydrase (CA)	0.560	29	4.462
Haemoglobin (Hb.)	0.690	19	4.279
Lysozyme (L)	0.786	14.3	4.155

peptide segregation. The vaccinal strain of IBDV presented six peptide bands of MW 20.3, 26.0, 32.0, 40.8, 62.0 and 90.9 KDa (lane 1). Another vaccine rendered three polypeptides with MW 40.8, 62.0 and 90.9 KDa (lane 2). Lanes 3 and 4 presented similar peptide pattern, both contained two peptide bands having MW of 40.8 and 90.9 KDa. In lane 2, two peptide bands and in lanes 3 and 4, three peptide bands were missing.

DISCUSSION

Infectious bursal disease (IBD) has become more significant due to huge economic losses inflicted over the past few years. Some antigenic variation exists between the strains of infectious bursal disease virus (IBDV) due to variable cleavage of precursor proteins (Jackwood *et al.*, 1984). Not much established work has been conducted regarding monitoring of antigenic

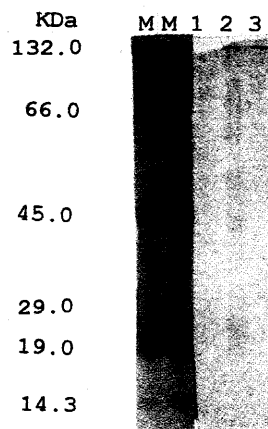


Plate 1: SDS-PAGE analysis of unsonicated field IBDV along with protein markers.

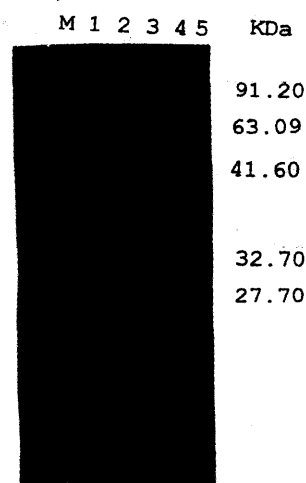


Plate 2: Electrophoretogram of field IBDV (Sonicated).

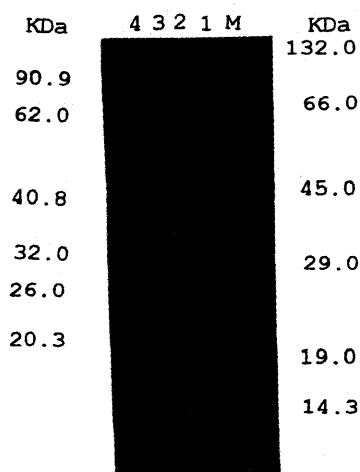


Plate 3: Polypeptide mapping of vaccinal strains of IBDV.

variation among various field isolates of IBDV in Pakistan. This could be one of the possible causes of the persistence of disease despite intensive vaccination program followed far and wide.

In the present studies sonication was done of some field isolates whereas some were used unsonicated. All the five field isolates generated five peptide bands (VP1, VP2, VP3, VP4 and VPX) with MW ranging from 27.7-91.2 KDa comparable with those reported by Fahey *et al.* (1985) but in case of unsonicated samples, no peptide band was observed. This indicates that sonication is necessary for the release of IBDV from the bursal cells (Ture and Saif, 1992).

Four vaccinal strains of IBDV tested in the present study showed different segregation pattern of peptides on SDS-PAGE. Out of four, only one vaccine contained all the five peptide bands comparable with those observed by Becht *et al.* (1988). The peptides of MW 40.8 and 90.9 KDa were found to be present in all four vaccinal strains (plate 3) that resembled in MW with VP3 AND VP1 viral proteins, respectively, as described by Muller and Becht (1982). The peptide band of MW 62.0 KDa was found to be present in lanes 1 and 2 and absent in other two lanes i.e., 3 and 4 which resembled the VPX protein in its electrophoretic mobility as reported by Azad *et al.* (1985). The peptide bands of MW 32.0 and 26.0 KDa were missing in three vaccinal strains and were present in only one which are comparable with pattern of VP2 AND VP4, respectively, as is also mentioned by Azad *et al.* (1985). Furthermore, it has been described by Fahey *et al.* (1985) that the peptide band having 32 KDa (VP2) MW is the major immunogenic polypeptide fraction of IBDV. It was evident that VP2 was presented by only one vaccinal strain of IBDV and was missing for other three vaccines. It may, therefore, be concluded that the non existence of this major immunogenic viral protein in most of the commercial vaccines of IBDV might have been the cause of the failure in getting protective immunity in flocks following IBD vaccination. A rigorous surveillance of the prevalent IBDV vaccinal strains through polypeptide mapping analyses would help developing complementary IBD vaccines to better combat overwhelming challenges.

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