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# STRUCTURAL PROTEINS OF FIELD ISOLATES OF INFECTIOUS BURSAL DISEASE VIRUS*

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Infectious bursal disease virus (IBDV) is the causative agent of Gumboro disease of chickens. This virus has a selective tropism for cells of Bursa of Fabricius. Economic losses in poultry industry can result from high mortality rates due to an acute course of the disease or from consequences of a B cell dependent immunodeficiency. Vaccination is thought to be the principal method of controlling this disease.

Recently outbreaks have been reported in vaccinated flocks. This lack of protection could be due to viral mutation resulting from persistence of the virus in the same locality and vaccination pressure. This may lead to increased virulence or failure of the field strain to get neutralized by antibodies to the vaccine strain. It is known that protein analysis of the virus by polyacrylamide gel electrophoresis (PAGE) can identify antigenic variants among different isolates. Taking this fact into account a study was undertaken to identify and compare the structural proteins of field isolates and a commercial vaccine strain of IBD virus.

## Materials and Methods

### Virus

Four field isolates and a vaccine strain were used for protein analysis by PAGE. Two of these isolates (PKD and EKM) were from vaccinated flock and other two (THA and KAN) from vaccinated flocks. The vaccine strain (VAC) was a commercially available vaccine.

### Propagation

All the five isolates were propagated in five week old sero negative chicks. The bursae collected on the third day of infection, homogenized to get 50% (w/v) suspension in PBS, and cleared by chloroform extraction was used for further purification and concentration.

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### Purification and concentration

The clear bursal suspension was centrifuged at 15,000 x g for 15 minutes at 4° C and the supernatant was treated with 8% polyethylene glycol (PEG) 6000 overnight with constant stirring to precipitate the virus and pelleted by centrifugation at 10000 x g for 30 minutes. The pellet was resuspended in PBS to get 1/10th of the original volume and was cleared by centrifugation. The clear supernatant was dialysed against PBS at 4° C to remove traces of PEG with frequent changes of PBS. After dialysis the clear fluid was centrifuged at 40000 RPM in Servo Combi Plus No.80 rotor for three hours at 4° C. The partially purified virus pellet was dissolved in 200 $\mu$l of TNE buffer (0.01M Tris-Hcl-pH 7.5; 0.15 M Nacl; 0.001M EDTA) and centrifuged at 10000 x g for 15 minutes to remove coarse particles by sedimentation. The protein concentration was assayed by Lowry's method (Lowry *et al.*, 1951) and was adjusted to 1mg/ml.

### Sample preparation

Equal volumes of sample and sample buffer (Tris 0.515 g; SDS 0.2 g; glycerol 1 ml and 0.5 ml of 2-mercaptoethanol) were mixed and heated in a water bath at 90° C for 1 hr.

Bovine serum albumin and chymotrypsin were also prepared similarly and used as markers.

### SDS-PAGE

The procedure described by Laemmli (1970) was followed in 16 x 20 cm glass plates.

Nine percent resolving and 5% stacking gel was prepared with six slots. Each well was loaded with separate samples. Simultaneously BSA and chymotrypsin were also loaded as marker. Electrophoresis was initially done at 110 V till the dye reached the surface of resolving gel and then the voltage changed to 150V and was continued till the dye reached the bottom of the gel. The gel was then removed and stained overnight with coomassie blue and then destained. The protein bands were read on a transilluminator.

### Results and Discussion

A total of 12 polypeptides were resolved on the gel and were designated serially as P1, P2, P3...P12. The molecular weights of the proteins were determined by comparing the distance migrated by known molecular weight proteins (BSA and chymotrypsin).

All the four field isolates resolved nine polypetides (Fig.). The molecular weights of all the proteins from different isolates were also the same. They were 86 kD (P2), 77kD (P4), 73 kD (P5), 62 kD (P6), 52 kD (P7), 47 kD (P8), 39 kD (P10), 36 kD (P11) and 32 kD (P12).

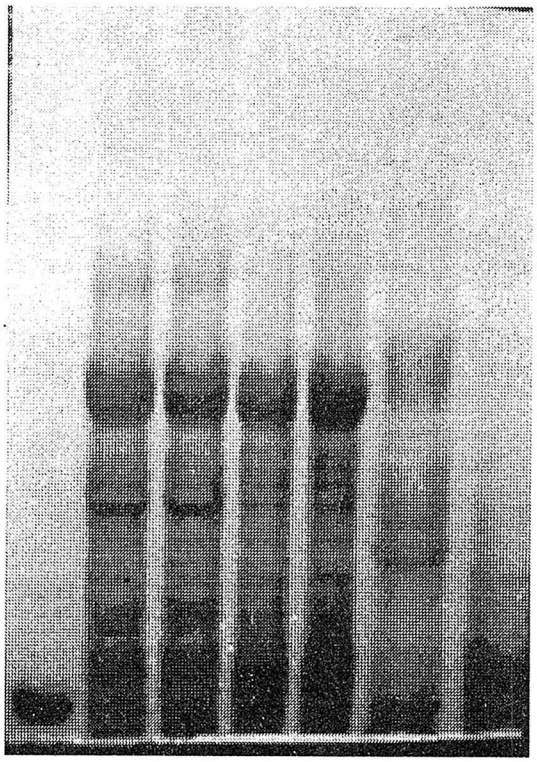

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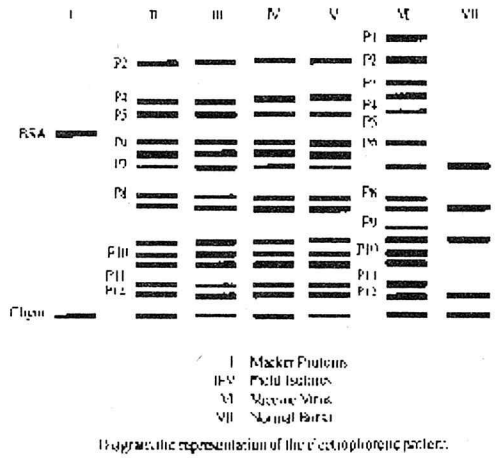
Fig. Electrophoretic pattern of IBDV isolates




Diagramatic representation of the electrophoretic pattern

The vaccine strain resolved all proteins with the following mol. wts., 93 kD (P1), 86 kD (P2), 80 kD (P3), 77 kD (P4), 73 kD (P5), 64 kD (P6), 47 kD (P8), 43 kD (P9), 39 kD (P10), 36 kD (P11) and 33 kD (P12).

Unlike the observations made in this study, Nick *et al.* (1976) demonstrated four polypeptides using the same system for protein analysis. The molecular weight of these proteins were 110 kD, 50 kD, 35 kD and 25 kD respectively for VP1, VP2, VP3 and VP4. The number and mol. wts. of proteins of IBD resolved by other workers also varied. Kher (1988) resolved seven polypeptides with mol. wts. of 82 kD, 74 kD, 44 kD, 37 kD, 30 kD, 28 kD and 26 kD. The seven polypeptides resolved by Vijayapraveen *et al.* (1995) from IBDV had 97 kD, 56 kD, 53 kD, 50 kD, 45 kD, 29 kD and 25 kD as their mol. wts. Variation in the number and mol. wts. of polypeptides of IBDV resolved by various workers can be attributed to the change in the methodology adopted, source of the sample and degree of purification.

In the present study the vaccine strain resolved 11 polypeptides as against nine in the field isolates. The proteins that were absent in the field isolates were P1 (93 kD), P3 (80 kD) and P9 (43 kD). It can also be seen from the figure that protein P7 (52 kD) is missing in the vaccine strain. Similarly a difference in the mol. wts. of P6 and P12 of the field isolates and the vaccine strain was also detected. However, all the field isolates and the vaccine strain had the 47 kD protein which is considered to be the protective antigen of IBD virus.

**Summary**

Four field isolates, two each from vaccinated and unvaccinated flocks and a vaccine strain of infectious bursal disease virus were concentrated and purified by initial PGE precipitation and subsequent differential centrifugation. The structural proteins of these isolates were resolved by SDS-PAGE using bovine serum albumin and chymotrypsin as molecular weight markers. All the four field isolates resolved nine polypeptides ranging between 32 kD to 86 kDm while the vaccine strain had 11 protein components the molecular of which ranged between 33 kD and 93 kD. The field isolates lacked the 93 kD, 80 kD and 43 kD proteins of the vaccine strain. The protein with mol. wt. of 52 kD was absent in the vaccine strain. A difference in the mol. wts. of proteins P6 and P12 of the field isolates and the vaccine strain was also detected.

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