

Adaptation of a Local Wild Infectious Bursal Disease Virus on Chicken Embryo Fibroblast Cell Culture

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ABSTRACT

A local very virulent infectious bursal disease virus (vvIBDV) was successfully adapted to grown on chicken embryo fibroblast (CEF) cell culture with concurrent loss of pathogenicity in specific pathogen free (SPF) chicks. The IBDV was isolated from field outbreaks and identified through agar gel precipitation test (AGPT) and counter current immunoelectrophoresis (CCIE) using specific antiserum. The positive samples (Bursae) were pooled and IBDV was purified through chloroform treatment and sucrose gradient centrifugation technique. The chick embryo infectivity (EID₅₀) of the purified vvIBDV was $10^{4.84}$ mL⁻¹. The virus was then passaged on CEF cell culture. It was completely adapted to grow in CEF cell culture after thirteenth passage. With reverse passive haemagglutination (RPHA), the titre of adapted virus was found as 1:1024 at fifteenth passage with complete loss of pathogenicity.

Key Words: Infectious bursal disease virus; Chicken embryo Fibroblasts (CEFs); Adaptation; Reverse passive haemagglutination (RPHA); vvIBDV

INTRODUCTION

Poultry industry is progressing rapidly under the shelter of Livestock sector of Pakistan, with the contribution of 11.4% of value addition capacity in the GDP (Anonymous, 2004). A part from management factors there are infectious diseases which play an important role in the economic losses of poultry industry. Infectious Bursal Disease (IBD) is still ranked at the top of list among the major viral infections in the country. IBD is a globally occurring, highly contagious, acute, viral disease of poultry. Although turkeys, ducks, guinea fowls and ostriches may be infected, clinical disease occurs solely in chickens. Only young birds are clinically affected. Infectious Bursal Disease virus has recently classified as a member of the genus Avibirnavirus of the family Birnaviridae (Dwight & Zee, 1999). Cosgrove first reported it in 1962, affecting chickens on a farm in Gumboro, Delaware, U.S.A (Cosgrove, 1962). Thus Gumboro disease became synonymous for the condition. The virus grows rapidly in the Bursa of Fabricius (BF) and the disease is characterized by acute onset, short course and cause extensive destruction of B-lymphocytes (Reddy *et al.*, 1997). Severe acute disease in 3 to 6 week-old birds is associated with high mortality, but a less acute or sub-clinical disease is common in 1 to 3 weeks old birds. The economic importance of this disease stems from heavy morbidity and mortality, reduction in growth rate and immuno-suppression making the birds susceptible to other diseases such as Newcastle disease (Shome *et al.*, 1997).

Non-vaccinated IBD flocks suffered more than

vaccinated flocks in field outbreaks of IBDV (Anjum *et al.*, 1993). Most of the IBDV vaccine showed variable degree of pathogenicity and also immunosuppressive effect (Hussain *et al.*, 2001). Studies have revealed that commercially available IBD vaccines have protein electrophoresis pattern different from the virus isolated from local outbreaks of IBD (Anjum *et al.*, 1996).

Application of chicken embryos has its own potential as some of the variant strains may not grow well through chorioallantoic membrane route. However, many continuous cell lines are reported for the propagation of IBD virus with variable success, but these cell lines have cancerous gene potential which may limit the product with lots of biological risk in the birds. Chicken fibroblast cell cultures were obtained from SPF eggs and used in the present study. Preliminary efforts were found successful in the adaptation and characterization of vvIBDV on primary cell culture system.

MATERIALS AND METHODS

Source of virus. Very virulent IBD virus (vvIBDV) locally identified, characterized and later egg adapted strain was procured from Department of Veterinary Microbiology. After propagation the virus was repurified through ultra centrifugation for 6 h by sucrose gradient method at 4°C (Zahoor *et al.*, 2005).

Pathogenicity of IBDV. Ten fold serial dilution of purified indigenous vvIBD strain was inoculated (0.2 mL) in eight groups (10^2 - 10^{10}) of 9-days old SPF embryonated eggs (ten eggs in each group). Morbidity and mortality percentage

was recorded up to 48 h post-inoculation and pathogenicity index in terms of chicken embryo infective dose (EID₅₀) was calculated.

Inoculation and adaptation of IBD virus. Chicken embryo fibroblasts were grown according to the method developed by Rebecca (1998). Each culture flask containing homogenous confluent layer of adherent chicken embryo fibroblast cells, was inoculated with 0.1 mL indigenous purified and recharacterized vvIBD virus. Flasks were gently shaken and incubated at 37°C and plaque morphology and plaque forming units (PFU) per microscopic field (MF) were recorded.

Reverse Passive Haemagglutination (RPHA) Test

Confirmation of the virus. The hyper-immunized antisera was raised against vvIBD virus strains in rabbits with the similar schedule of multiple inoculations of IBD vaccinal virus strain of D-78. The presence of virus in the chloroform treated filtered clarified fluid was checked through the standard technique of counter current immunoelectrophoresis (CCIE) as detailed by Hussain *et al.* (2004).

Test procedure. Ten ml of human blood group “O” (-ve) was collected aseptically and centrifuged at 300 xg for 15 min. The packed erythrocytes were resuspended in sterile physiological saline (0.89% NaCl) and the cells were given three washings. The washed human group “O” erythrocytes were sensitized with known antibodies (1/10 dilution of antiserum against vvIBD virus) and were washed thrice in PBS and the sensitized erythrocytes suspension was maintained as 2% concentration in PBS.

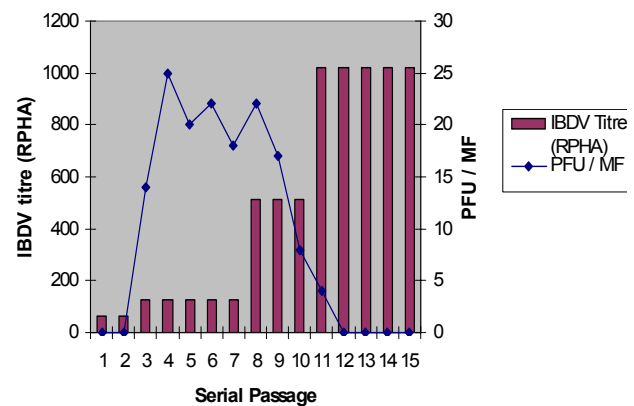
Two fold serial dilution of vvIBD virus suspension was prepared in PBS and mixed with equal volume (50 µL) of sensitized RBC (2%). After incubation at 35°C for 30 min the haemagglutination titre was recorded as described by (Rejeswar & Dorairajan, 1999).

RESULTS

Confirmations and purification of virus. Bursal cell suspension supernatants and the purified IBDV was confirmed through the visibility of clear white single precipitation line in twenty hours time period, thus indicating the homologous antigen and antibody system and no line was present between the negative control well and antibody as detected through AGPT.

Adaptation of IBDV on chicken embryo fibroblast (CEF) cell culture. The IBDV was serially passaged on CEFs up to 15th inoculations. After each inoculation and passage IBDV was observed for the typical CPEs. In the first two passages no lesions of virus were observed. From passage number three to passage number seven; typical CPEs (rounding of fibroblast cells) were recorded. CPEs were found increasingly till passage number nine. Later, from passage number ten CPEs started to reduce in intensity along with small plaques converted into comparative wider diffuse edges plaques till passage number thirteen. Passage

Fig. 1. Results of IBDV titre and plaque forming units obtained at different passage in chicken embryo fibroblast cell culture



fourteenth and fifteen showed homogenous rounding throughout the flask without any typical plaque demarcation.

Antigen titer in different passages through RPHA. In the first two passages very low quantity (1:64) of virus was detected but it gradually increased to 1:128 until 7th passage followed by 1:512 as the passages were performed up to 10th then it became constant (1:1024) up to the 15th one (Fig. 1). The supernatant culture fluid showed positive results with regards to the confirmation of IBD virus through CCIE.

DISCUSSION

The preliminary efforts were found successful to attain adaptation of local field isolate of vvIBDV on chicken embryo fibroblasts CEF cell culture. A standard procedure was adopted for processing of bursa for virus isolation as suggested by Rosenberger *et al.* (1998). The suspension supernatant fluid was continuously monitored for the confirmation of IBDV through agar gel precipitation test (AGPT) and counter current immunoelectrophoresis (CCIE), using known hyper immune serum. Lukert and Saif (2003) also described AGPT for detection of IBDV from bursa of Fabricius. Muhammad *et al.* (1995) quantified the IBDV in infected birds and embryonated chicken eggs through agar gel precipitation assay. Ahsan *et al.* (2002) and Ahmed *et al.* (2004) also used AGPT for confirmation of IBD and ND viruses.

The positive bursal suspension samples were pooled together and processed further. After the homogenate clarification the supernatant was subjected to chloroform treatment of virus, three distinct layers were obtained after centrifugation. The top clear fluid contained IBDV, middle layer having bursal tissue debris and bottom layer constituted of chloroform as obtained by Reddy *et al.* (1997); Anjum *et al.* (2001) and Ahmad *et al.* (2004). Further purification was conducted through velocity density gradient centrifugation technique and confirmed by CIE. The Pathogenicity index in the form of chicken embryo

infective dose (EID₅₀) was 10^{4.84} mL⁻¹ in our study. The range of lethality of IBDV adopted by various workers was around 10⁴ mL⁻¹ as detailed by Ahad (2002), Lee and Lukert (1986), McNulty *et al.* (1979).

In the first two passages no lesions of the growth of virus were observed. From passage number three to passage no. seven, typical CPEs (Rounding of Fibroblast cells) were recorded. Small typical CPEs were found increasing till passage number nine. Later on from passage number ten CPEs started to reduce in intensity and were absent till passage number thirteen. Whereas, overall rounding of fibroblast cell were visible throughout without any noticeable separate cluster of typical CPEs in the monolayer till 15th passage. The findings were found more closely related to the pattern as observed by Rinaldi *et al.* (1972) and Cho *et al.* (1979).

In first two passages very low quantity (1:64) of virus was detected but it gradually increased (1:1024) as the passages were performed up to 11th then it remained persistent till the 13th one. The passage no. 13 showed no CPEs, which may indicate that the locally isolated vvIBDV has attained adoptability through serial passages in chicken embryo fibroblast cell culture. There was corresponding increase occurred in the concentration of IBDV and the pathogenicity index also reduced to 10^{1.6} mL⁻¹ at 13th passage. Therefore the virulence of IBDV was lost during the adaptation on CEF cell culture but antigenicity was retained. Similar loss of virulence in IBD virus upon adaptation was also reported by Khan (2005) and Yamaguchi *et al.* (1996).

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