

Isolation and characterization of a field isolate of infectious bursal disease virus in BGM-70 cells

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Infectious bursal disease (IBD) is an acute, highly contagious viral infection of growing chickens caused by a virus belonging to the genus *Avibirna* virus of the family *Birnaviridae*. Since re-emergence of very virulent form of IBD during 1990s, the virus has been continuously isolated from unvaccinated as well as vaccinated flocks in India (Rao *et al.* 2001, Gaba *et al.* 2007, Mor *et al.* 2010). Isolation of virus from vaccinated flocks warranted isolation and characterization of field isolates of IBD. Keeping this in view, present study was undertaken to isolate IBDV from an outbreak.

Sample: An outbreak was reported at Jagdishpur village of Sultanpur district in Uttar Pradesh in IBD vaccinated broiler farm comprising 2000 birds. The clinical and postmortem examination was conducted in ailing and dead birds. The clinical pictures as well as postmortem findings were suggestive of IBD. Mortality rate was 14%. Fifty representative samples of bursa were collected from dead and ailing birds showing specific clinical signs and lesions of IBD and brought to laboratory on ice.

Antisera: Hyerimmune serum of IBD virus available in the Department was used. It was raised in cockerels using cell culture adapted 'Georgia' (Intermediate) strain commercial vaccine of IBD. The serum was inactivated at 56°C for 30 min before use.

Preparation of the sample: The samples of bursa were titrated and a 50% (w/v) suspension was prepared in phosphate buffer saline (PBS). The resultant material was subjected to 3 cycles of freezing (at –20°C) and thawing followed by centrifugation at 10 000 rpm for 10 min. The supernatant was collected, treated with penicillin (@100 IU/ml) and streptomycin (@100 mg/ml) and tested for IBDV antigen in agar gel immunodiffusion (AGID) test as per Hirai

et al. (1972). The samples showing positive AGID were further diluted to give a final concentration of 10% (w/v) of the tissue, filtered through 0.22 µm membrane filter and used as inoculum for virus isolation in cell culture.

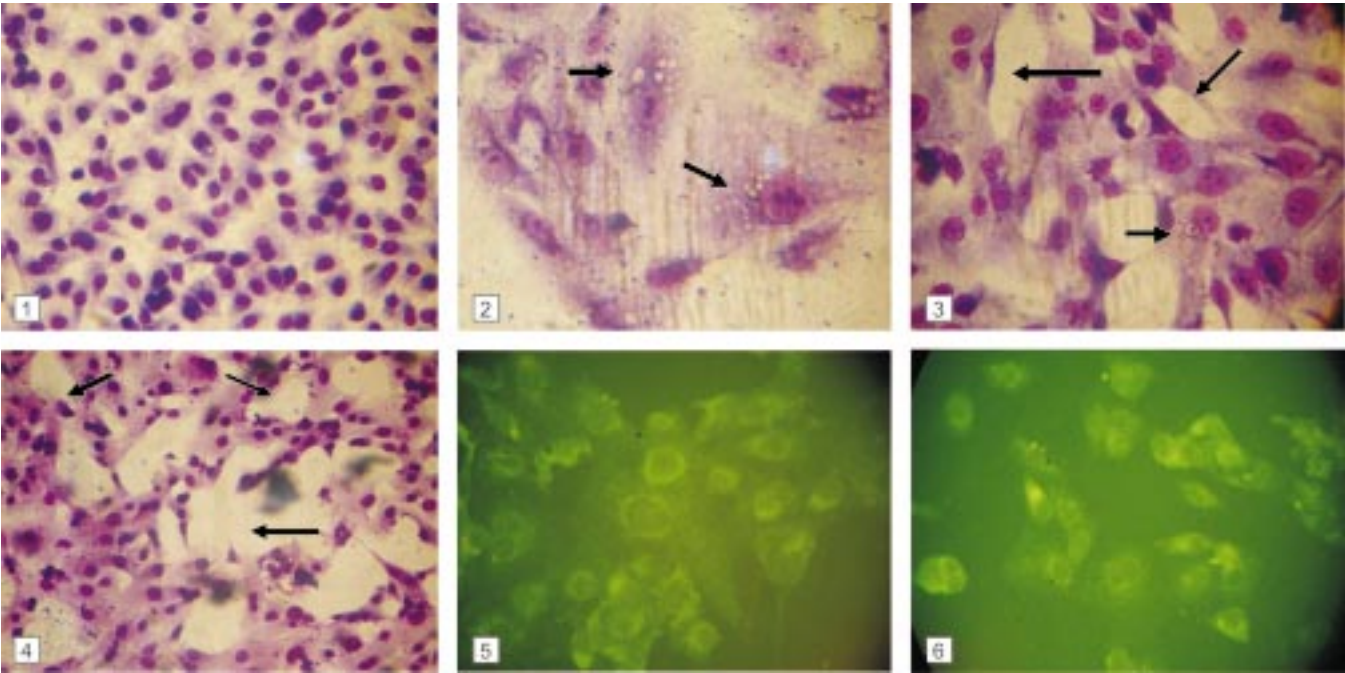
Cell line: BGM-70 (Baby gravid monkey kidney) cells procured from Department of Veterinary Microbiology and Immunology, College of Veterinary and Animal Sciences, G.B.Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), was maintained by regular sub-culturing in Dulbaccos Modified Eagle's medium (DMEM) supplemented with tryptose phosphate broth (TPB)(0.4% w/v) and Fetal bovine serum (5%v/v).

Adaptation of IBDV: The field IBDV was serially passaged 10 times in BGM-70 cells. The cytopathic effects (CPE) were recorded in unstained preparations at the end of each passage. The coverslip cultures infected with field isolate of IBDV were also stained with May-Grunwald and Giemsa (MGG) stain (Merchant *et al.* 1960) to study the CPE. The specificity of cytopathic effects was checked by detecting viral antigen in the infected cell monolayers on sixth day at each passage using indirect FAT (Tsai and Saif 1992). The cell culture supernatant collected at the end of each passage was tested for IBDV antigen using AGID (Hirai *et al.* 1972), counter-current immunoelectrophoresis (CIE) (Berg 1982) and indirect ELISA (Jackwood *et al.* 1982). The infectivity titre (TCID₅₀/0.1 ml) of the virus was calculated from fourth passage onwards using micro titration method in BGM-70 as per Reed and Munch (1938).

The field isolate of IBDV did not show any apparent cellular changes up to second passage in the unstained monolayers. At third passage level, the changes became appreciable with increased cellular granularity, rounding and clumping at 120 h PI. These changes further intensified and were seen at 108 h PI in the fourth passage. At eighth passage, the CPE were observed as early as 48 h PI, which were characterized by marked degeneration and detachment of cells leading to formation of micro- and macro-plaques. These changes persisted up to tenth passage. The study

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Figs 1–6. 1. Uninfected BGM- 70 cells. 2. IBDV infected BGM-70 cells showing cytoplasmic vaccuolation (MGG 40 ×). 3. IBDV infected BGM-70 cells showing intracytoplasmic inclusions and micro plaques (MGG 40 ×). 4. IBDV infected BGM-70 cells showing micro- and macro-plaques (MGG 10 ×). 5. IBDV infected BGM-70 cells showing perinuclear fluorescence (FAT 40 ×). 6. IBDV infected BGM-70 cells showing diffused cytoplasmic fluorescence (FAT 40 ×).

revealed that two blind passages were needed to allow the adaptation of virus in BGM-70 cells. Toroghi *et al.* (2001) reported that up to 4 blind passages were required for isolation of IBD virus in CEF and BGM-70 cells. Gaba *et al.* (2007) also reported first appearance of CPE at 72 h PI at third and fourth passages, respectively, in 2 different field isolates of IBD virus. However, Reddy and Silim (1998) reported that the field isolate could readily replicate in healthy BGM-70 cells without the necessity of adaptation by blind passages. Although in the stained preparations, CPE were seen at few places even at second passage but characteristic changes like rounding, clumping and the granulation were well appreciable from third passage onwards. At fourth passage, the monolayers showed generalized CPE such as extensive cellular rounding and clumping at 48 h PI, cellular vacuolation at 120 h PI (Fig. 2), formation of micro plaques at 96 h PI, macro plaques at 144 h PI and intracytoplasmic inclusion bodies at 144 h PI (Figs 3, 4). With increase in passage level, these changes became more pronounced and appeared more readily. At eighth passage level, the virus appeared to be stabilized in BGM-70 cells as all these changes were consistently seen. The CPE started at 24 h PI and the cells showed multiple inclusions. The degenerative changes like vacuolation, detachment and sloughing of monolayers became evident from 96 to 120 h PI. There are several reports of IBDV replication in BGM-70 cells (Toroghi *et al.* 2001, Rao *et al.* 2001) indicating that CPE were more pronounced in BGM-70 cells in comparison to others. In present study,

the infected monolayers invariably exhibited yellow green fluorescence at the perinuclear area in the cytoplasm of the infected cells starting from 48 h PI at second passage (Fig. 5). Almost entire cell sheet appeared involved at 96 h PI at fifth passage onwards as the cells exhibited diffused fluorescence throughout the cytoplasm (Fig. 6).

The infectivity titre of field isolate of IBDV in BGM-70 was $2 \times 10^{11.8}$ TCID₅₀/ml at fourth passage, which increased gradually insubsequent passages to reach up to $2 \times 10^{15.74}$ TCID₅₀/ml at tenth passage (Table 1). The quantitative estimation of IBDV antigen was done at different passage

Table 1. ELISA and infectivity titers of IBD virus in BGM-70 cells

Passage No.	ELISA titers (log ₁₀ /50 µl)	Virus infectivity titers (TCID ₅₀ /ml)
P ₁	ND*	ND*
P ₂	2.33	ND*
P ₃	5.00	ND*
P ₄	6.33	$2 \times 10^{11.8}$
P ₅	7.67	$2 \times 10^{12.38}$
P ₆	8.33	$2 \times 10^{12.68}$
P ₇	9.66	$2 \times 10^{13.79}$
P ₈	9.67	$2 \times 10^{14.26}$
P ₉	10.33	$2 \times 10^{15.54}$
P ₁₀	10.67	$2 \times 10^{15.74}$

*ND, Not done.

using liquid phase ELISA, and it was found that ELISA antigen titer were increased from $2.33 \log_{10}/50 \mu\text{l}$ at second passage to $10.67 \log_{10}/50 \mu\text{l}$ at tenth passage (Table 1). Marquardt *et al.* (1980) found ELISA 20 to 25 times more sensitive than AGID. Mathur *et al.* (1999) performed AGID and ELISA to ascertain the presence of IBDV during adaptation of K-2 strain of IBDV in CEF cell culture.

In AGID, the concentrated cell culture supernatant of infected BGM-70 cell monolayers showed 2 precipitation lines with hyper immune sera raised with 'Georgia' strain. Hirpurkar (1995) also observed similar findings using field isolate while Cullen and Wyeth (1975) observed 2 to 3 precipitation lines in strong reaction of AGID test. The cell culture supernatants collected at various passages were also found positive for IBDV antigen in counter immunoelectrophoresis. Kadam and Jhala (2003) found that the sensitivity of AGID was 78.26% and specificity 100% relative to CIE and dot ELISA while overall agreement of AGID with CIEP and dot ELISA was 83.33%.

SUMMARY

The infectious bursal disease virus (IBDV) from a field outbreak was isolated and characterized in BGM-70 cells. The growth and adaptation of virus was assessed by study of cytopathic effects, infectivity titers and ELISA titer. After 2 blind passages, the isolate produced characteristic cytopathic effects (CPE), viz. rounding and clumping of cells, increased cellular granularity and formation of micro- and macro-plaques in unstained and intracytoplasmic inclusions and vacuolation in stained monolayers. At eighth passage level, the virus appeared to be stabilized in BGM-70 cells as all these changes were consistently seen in subsequent passages. The presence of intracellular virus antigen was detected by immunofluorescence test (FAT). The IBDV antigen was detected in the cell culture supernatants at each passage by agar gel precipitation test (AGID), countercurrent immunoelectrophoresis (CIE) and enzyme linked immunosorbant assay (ELISA).

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