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Short Communication

Adaption of Infectious Bursal Disease Virus (IBDV) on Chicken Embryo-fibroblast Culture

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Infectious bursal disease virus (IBDV) from the local outbreak in poultry flocks was purified and confirmed through Agar gel immune-diffusion test (AGID). Purified IBD virus was attenuated by serial passages in eggs through chorioallantoic membrane (CAM) route. The mortality was up to 70% in the first 6 passages and reduced from 60 to 0% up to the 16th passage. No mortality was observed between 16th-24th passages. Chicken embryo fibroblasts were prepared from 9 days-old specific pathogen free (SPF) embryonating eggs and then infected with purified IBDV. Typical cytopathic effects (initiated from star-shaped cells which led to rounding of fibroblast cells) were observed on inverted microscope. This approach will help to produce cost effective live-attenuated infectious bursal disease vaccine from local strains on chicken embryo fibroblasts cells.

Key words: Infectious bursal disease virus (IBDV), Agar gel immunodiffusion test (AGID), chorioallantoic membrane, chicken embryo fibroblast, specific pathogen free (SPF).

INTRODUCTION

Infectious bursal disease (IBD) is severe acute disease of 3-6 week old birds, which causes lymphoid depletion of the bursal, resulting into significant depression of the humoral antibody response (OIE, 2008). The disease was firstly reported in chickens by Cosgrove on a farm in Gumboro, Delaware, U.S.A (Cosgrove, 1962). Two distinct serotypes (1 and 2) of IBDV have been recognized. The serotype 1, which displays a wide variation in pathogenic potential, is virulent for chickens, whereas serotype 2 is virulent for turkeys (Jackwood and Saif, 1987). Chickens affected by the variant strains are characterized by severe atrophy of the bursal without showing the inflammation associated with infection by classical strains (Vakharia et al., 1994). Highly virulent IBD virus induces severe clinical signs followed by death, with severe damage to the bursal, thymus and spleen. Classically virulent IBD virus induces low or no mortality, with severe damage to bursal (Lukert and Saif, 1997). It has been observed from the previous study that the growth of IBDV on embryonating eggs (ECEs) is satisfactory for the isolation of virus from clinical and sub-clinical cases (Aftab et al., 2010).

In the present study, local isolate of IBDV was attenuated on ECEs and adapted on primary chicken fibroblast. The purpose of this study was to produce cost effective live-attenuated infectious bursal disease vaccine on chicken embryo fibroblast cells from local strain.

MATERIALS AND METHODS

Collection and processing of sample

Bursal materials from the local outbreak of IBD in broiler chickens were chopped by using the scalpers and added 10% W/V phosphate buffered solution, containing antibiotics, that is, penicillin at 100 IU/ml, streptomycin at 100 µg/ml and gentamycin at 50 µg/ml. Homogenate was centrifuged at 5000 rpm for 20 min. The IBD virus was purified by ultra-centrifugation method.

Attenuation of IBDV

10 day old specific pathogen free (SPF) embryonating

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chicken eggs were procured from a private poultry hatchery. Purified IBD virus was attenuated in serial passages on eggs through chorioallantoic membrane (CAM) route. Eggs were incubated and observed daily for viability and lesions. Dead embryos during the first 24 h post inculcation were discarded. Mortality was recorded between 3-5 days post infection (PI). Chorioallantoic fluid (CAF) was centrifuged and supernatant was collected and stored at -20°C until used for laboratory test.

Establishment of primary chicken embryo fibroblast cells

Chicken embryo fibroblasts were prepared from 9 days-old SPF embryonating eggs as described by FAO (1996) with some modifications. Briefly, tissues were chopped by using the scalpers and washed with several changes of phosphate buffered saline solution until the supernatant fluid was clear. After, chopped tissues fragments were transferred to the trypsinization flask containing a magnetic bar and 0.025% solution of trypsin was added. The solution was stirred for 5 min and then the tissues were allowed to settle and the supernatant was discarded. Trypsinization process was repeated for 5 times. And then it was filtered through muslin cloth. Filtrate was centrifuged and supernatant was discarded. Cells were maintained in Glassgow minimal essential medium (GMEM) supplemented with penicillin, streptomycin and nystatin and 5% foetal calf serum.

Adaption of attenuated IBDV on chicken embryo fibroblast cells

Primary chicken embryo fibroblast cells were infected with serially chorioallantoic fluid passaged IBDV. The cytopathic effects were observed and IBDV was harvested and stored at -20°C until used for laboratory test.

Serological confirmation

The hyper immune serum was raised in Chinchilla rabbits against the local field isolate of IBD virus. Serum was inactivated at 56°C for 30 min and stored at -20°C. Detection of purified attenuated IBD virus was carried out by Agar gel immunodiffusion test (AGID) (OIE, 2008).

RESULTS AND DISCUSSION

Cells and virus were tested for sterility. Nutrient broth, thioglycolate medium and soybean casein digest medium were inoculated and found negative for any aerobic, anaerobic and fungal growth.

Purification and confirmation of IBDV

Ultra-centrifuged purified IBDV was confirmed by agar gel immunodiffusion (AGID) test. Clear precipitation lines were observed between the wells containing known IBD hyper immune serum and purified IBD antigen, suggesting the presence of IBD virus in the suspected samples. No line was observed between the negative control well and antibody as detected through AGID.

Pathogenicity study on chicken embryonated eggs

IBDV was adapted through serial passage in chicken embryonated eggs. Following the CAM inoculation, 100% mortality was observed. The mortality was up to 70% in the first 6 passages. The mortality was reduced from 60% to 0% up to the 16th passage. No mortality was observed between 16th-24th passages. Hemorrhages on the toes, legs and body were observed. In the early passages, the titer of the virus was lowered in CAF but serial passage increased the titer of IBDV.

Cytopathic effects on chicken embryo fibroblast

Already egg's adapted IBDV was successfully adapted to grow in chicken embryo fibroblast cell cultures showing cytopathic effects. In the first two passages, no pathogenic effects were observed on cell cultures but on-ward passages, typical CPEs (initiated from star shaped cells which led to rounding of fibroblast cells) were observed on inverted microscope.

Immuno-suppression in the chickens is due to the severe cellular damage in the bursal of fabricius. There is the activation of the complement system due to production of immune complexes between the IBD virus and antibodies in bursal, the result of which, there is cellular damage (Ezeokoli et al., 1990). In the present study, the available method for the detection of IBDV antibodies was AGID test, which measures antibodies to specific antigen (IBD strain D78). Adaption of IBDV in CEF cell culture at various passages levels has been reported by many scientists (Jackwood et al., 1987; Dash et al., 1991; Singh and Dhawedkar, 1992). It has been reported from the study that very virulent (vv) IBDV normally do not grow in cell culture (Müller et al., 2003); but by using site-directed mutagenesis and the reverse genetics approach, some scientists adapted the vv IBDV on chicken embryo cell cultures. Repeated passages of classical virulent (cv) IBDV in tissue culture at high MOI led to attenuation and the formation of plaque phenotype, which is used as live vaccine (Müller et al., 1986). So, in the present study, field isolates might belong to cv IBDV strain. Clinical picture and the course of the disease usually are indicative of an IBDV infection. However, pathological lesions and histo-pathological investigations combined with the demonstration of viral antigens by AGID test are confirmative to IBD. It has also been observed that as the number of passages increased, mortality pattern and lesions on body surface of chicken embryo were reduced, so indicative of low pathogenicity.

In conclusion, local isolates of IBDV successfully attenuated in embryonating chicken eggs (ECEs) and then adapted to chicken embryo fibroblast cell cultures. This approach will help to produce cost effective live-attenuated infectious bursal disease vaccine from local strains on cell culture.

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