

Short Communication

Identification of infectious bursal disease virus (IBDV) through agar gel immunodiffusion (AGID) test and its purification by ultracentrifugation

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Infectious bursal disease (IBD) is a highly important immunosuppressive disease of young chickens caused by infectious bursal disease virus (IBDV). Infected bursae were collected from filed outbreaks of IBD at Lahore, Pakistan. A 10% W/V suspension was made in the phosphate buffer saline and centrifuged at 5000 rpm for 20 min. The presence of IBDV in the supernatants of suspect homogenates was checked through agar gel immuno-diffusion test. The collected material was given serial passage in 10 days old embryonating eggs at an inoculation rate of 0.2 ml/egg through chorio-allantoic membrane (CAM) route. The material collected at different passages was centrifuged twice at 20000 rpm for 45 min to remove tissue debris. The clear supernatant was subjected to ultracentrifugation at 49500 × g for 6 h at 4°C. The purified IBD virus was obtained in pellet form and these pellets were disrupted in TNE buffer to be stored at -70°C till further use. The study suggested that the bursa of fabricius is the best tissue source for the isolation of virus.

Key words: Young chicken, infected bursae, isolation of IBD virus, agar gel immuno-diffusion (AGID), ultracentrifugation.

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious disease of young chicken that primarily affects the bursa of fabricius leading to immunosuppression (Taghavian et al., 2013). The disease was first recognized as a specific disease entity by Cosgrove in 1962. It was shown to cause immuno-suppression and increase susceptibility of chicken to other diseases (Faragher et al., 1972).

IBD virus (genus: *Avibirnavirus*; family: *Birnaviridae*) is an immunosuppressive virus and causes significant morbidity and mortality in young chickens. The stability of IBD virus in the presence of disinfectants makes it serious threat to the young chickens (Muller et al., 2012). The IBD virus is transmitted by oral-fecal route (Muller et al., 2003, 2012). The IBD virus has two serotypes, although only serotype-I is pathogenic in chickens. Within serotype-I, classical and antigenically distinct variant strains can be differentiated. Since the late 1980s in

Africa, Asia and Europe “very virulent” (vv) strains have been found circulating and were antigenically similar to the classical strains (Saif and Lukert, 1997).

The recognition of immunosuppressive effect of IBD virus has greatly increased the interest of researchers in the control of this disease. Due to the immunosuppressive effect of IBD, this disease is recognized with heavy economic losses in commercial poultry farming (Roslaes et al., 1989).

Keeping in view the importance of this disease and possibility for the presence of variant strains, the present study was planned for the isolation, identification and

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purification of local isolates of IBD virus.

MATERIALS AND METHODS

The bursae of fabricius of suspected IBD cases from local outbreaks were processed as seen here under:

Processing technique

A 10% W/V suspension of infected bursae was made (Reddy et al., 1997) by chopping with a pair of scissors and grinding in a sterilized glass tissue grinder. The phosphate buffer solution containing antibiotics (100 IU penicillin-G per ml, 100 µg streptomycin per ml and 50 µg gentamycin sulphate per ml) was used. The suspension was centrifuged at 5000 rpm for 20 min and the supernatant was collected and stored at 20°C till further processing in embryonating eggs.

Confirmation of the virus

The presence of IBDV in clear supernatants of different passage levels in embryonating egg was checked through agar gel immunodiffusion test (AGID) against hyper-immune serum raised in rabbits against IBDV vaccine (D-78) according to Barnes et al. (1982).

Purification and concentration of field isolates of IBDV

The material of different passage levels, which was found positive through AGID test, was centrifuged at 20000 rpm for 45 min twice. The clear supernatant was centrifuged at 49500 × g for 6 h at 4°C. The pellets so obtained were disrupted in TNE buffer solution (Nobukhiko et al., 1995) and stored at 70°C till further use.

RESULTS AND DISCUSSION

IBD has become more significant due to huge economic losses inflicted over the past few years. IBD virus exists worldwide in at least two distinct serotypes I and II, although only serotype I is virulent for chicken. In the present study, the presence of IBD virus was checked through agar gel immunodiffusion test (Chullen and Wyeth, 1975) and field samples that were positive for IBD virus produced precipitin lines between central antiserum well and the surrounding samples' wells (Tsukamoto et al., 1995). As such, isolation of IBD virus may help us to prepare quality vaccine against IBD, which will protect our poultry against local strain prevailing in the country.

The results from this study suggest that the bursa of fabricius is the best tissue source for virus isolation. The embryonating eggs which were injected with virus showed embryonic death on day 4-6 post inoculation. However, embryonic death depends upon virulence of the strain and antibody titer of egg yolk (Hitchner, 1970).

The purification and concentration of IBD virus was carried out as described by Nobuhiko et al. (1995). The pellets so obtained were disrupted in TNE buffer solution and stored at -70°C for use in cell culture of embryonating eggs for virus propagation to prepare IBD vaccine from local IBD virus isolates.

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