

Studies on Infectious Bursal Disease Virus Isolated from Field Outbreaks

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ABSTRACT. *Out of forty five samples of bursae collected from dead or diseased chicken from various places, twenty four samples were identified as positive for infectious bursal disease (IBD) by agar gel precipitation test (AGPT) and immunofluorescent antibody test (IFAT). Passage of 20 bursal homogenates in embryonated chicken eggs showed very distinct lesions in embryos. Ten isolates from chick embryos were passaged in chick embryo fibroblast cell cultures and the cytopathic effect was detected in the 5th passage. AGPT's using ten bursal homogenates with known classical serotype 1 antigen and serotype 1 anti serum suggest that the field isolates are identical and probably belong to classical serotype 1.*

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious and immuno-suppressive disease of young chickens. In Sri Lanka, an outbreak of suspected IBD was reported in Vavuniya in 1995 (Anon, 1995). In 1996, an epidemic of IBD occurred in North Western and Western provinces. This had later spread to other neighbouring provinces. This communication describes the initial studies undertaken on isolation and identification of field viruses from these outbreaks.

MATERIALS AND METHODS

Collection of samples

Forty five samples of bursae of Fabricius were received by the Veterinary Research Institute from different areas of suspected outbreaks of IBD for confirmation.

Preparation of bursal homogenate

The samples of bursae were triturated separately to make 10% (v/v) in sterile phosphate buffer saline (PBS) (pH = 7.2) with added antibiotics. The homogenates were frozen and thawed 3 times and clarified by centrifugation at $1800 \times g$ for 15 minutes.

Serological identification by agar gel precipitation test (AGPT)

One percent (w/v) agarose in PBS (pH = 7.6) was boiled and 0.01% of sodium azide was added.

Agar plates were prepared using an offset linear pattern of wells with 6 mm diameter, 3 mm depth and 3 mm interspace. The wells in the outer 2 vertical rows were filled with bursal homogenates and the wells in centre row were filled with IBD virus serotype 1 antiserum obtained from SPAFAS, U.S.A. One of the wells in outer rows was filled with positive control IBD virus antigen obtained from CVL, Weybridge, U.K. Plates were incubated at room temperature in humid condition for 48 hours and examined. Precipitation lines were recorded within 48 hours and the samples positive for AGPT were passed through membrane filters of 0.45 microns pore size and stored at -40°C .

Isolation in chick embryos

Fertile chicken eggs were obtained from healthy flocks of Karandagolla white hens maintained at Karandagolla NLDB farm. Twenty bursal homogenates that were positive in AGPT were used for this purpose. Ten day old live embryonated eggs were inoculated with 0.1 ml of bursal homogenate *via* the chorio-allantoic membrane (CAM). For each bursal homogenate, 5 embryonated eggs were used. These inoculated eggs and uninoculated control eggs (5 in number) were incubated at 37°C for 7 days. The embryos dying after 24 hours or surviving up to 7 days post inoculation were chilled and opened for examination along with control embryos. The CAM, liver, kidney and spleen from the embryos were processed in the same way as bursal homogenate and used for further passage in embryos. At the 3rd passage, there were very distinct typical IBD virus lesions seen. Samples collected from those eggs that did not show distinct lesions were passaged up to 4 times. The presence of IBD virus at each passage was tested by IFAT and AGPT.

Virus isolation in tissue cultures

Using 10-day old chicken embryos obtained from the same source described earlier, primary and secondary chicken embryo fibroblast (CEF) cultures were prepared in polystyrene flasks. The medium used was M199 (Sigma). When the cultures were confluent, they were inoculated with the virus isolated in chick embryos and incubated at 37°C for 5 days. During incubation, cultures were examined daily for cytopathic effect. The cultures were disrupted by 3 cycles of freezing and thawing and clarified by centrifugation at 250×g for 10 min. These virus materials were stored at -40°C and used for further passages in CEF cultures. Up to 5 passages were carried out and the presence of virus at each passage was tested by IFAT and AGPT.

Immunofluorescent antibody test

Coverslip cultures of secondary chick embryo fibroblast (CEF) were grown in Leighton tubes. The cover slips were harvested 24 h after virus inoculation, washed in PBS, fixed in acetone for 5 min and stained with fluoresceine isothiocyanate immunoconjugate (Poultry Health Centre, Deventer, Netherlands) for 40 min at 37°C. The cover slips were washed with PBS, mounted in 50% glycerol and examined under ultra violet illumination.

AGPT for differentiation of IBD virus isolates

As described by Wyeth and Chettle (1988), AGPT was carried out for 10 samples of bursal homogenates and IBD virus classical serotype 1 antigen (strain - Cheville 1/68) (CVL, Weybridge) against serotype 1 antiserum produced by using the virus belonging to classical serotype 1 - strain 2512 (SPAFAS, U.S.A).

Agar plates were prepared as mentioned earlier. Bursal homogenates were filled in alternate wells in the outer two vertical rows. The remaining wells in the outer rows were filled with the known antigen. The wells in the centre row were filled with the antiserum. Plates were incubated at room temperature in humid condition for 48 h and examined.

RESULTS AND DISCUSSION

Agar gel precipitation test

The homogenate of bursae collected from field cases of suspected IBD showed distinct precipitation lines with reference to serotype 1 antiserum. These precipitation lines were continuous and the points of contact of these lines between wells were curved without producing any spurs.

In the other test, where the bursal homogenates and reference antigen were in alternate wells, there were distinct precipitation lines and these lines were found to be fused with the lines formed by reference serotype 1 antigen without any spurs. Wyeth and Chettle (1988) used AGPT to differentiate strains of IBD virus. Our observations indicate that these field isolates are IBD virus and that they are identical to serotype 1 group.

When samples collected from chick embryo passage were tested using AGPT, the precipitation lines were developed only after the 3rd passage. However, isolates from CEF cultures showed distinct precipitation lines after every passage.

Immunofluorescent antibody test

The virus isolates from chicken embryos and CEF cultures at each passage were confirmed by the examination of intracytoplasmic fluorescence for the presence of IBD virus.

Chick embryo passage

Very distinct lesions were observed at the 3rd passage. There was approximately 25% mortality in IBD virus inoculated embryos between 3 and 6 days of post inoculation. The lesions observed in dead and live embryos were dwarfism, abdominal and cerebral oedema, greenish discolouration and ecchymotic haemorrhages of liver and necrosis of kidney in some embryos. CAMS were thickened, congested and sometimes small opaque pocks were observed. Occasionally there were haemorrhages in the cerebral region and along feather tracts. The lesions were extensive when the passage level increased. These observations are in accordance with earlier report (Hitchner, 1970).

According to Lukert and Saif (1991), the variant serotype 1 IBD virus causes splenomegaly and liver necrosis with little mortality of embryos. Mekkes and Dewit (1994) stated that variant strains will not kill embryos. Our findings here suggest that the IBD virus isolates used in this study probably belong to classical serotype 1.

Passage in CEF

Cytopathic effect was observed at the 5th passage. This developed on the 3rd day of post inoculation as small round cells which detach from the monolayer. McNulty *et al.* (1979) recorded similar changes and claimed that IBD virus must be grown in chicken embryos first before being isolated in cell cultures.

CONCLUSIONS

Virulent IBD virus is probably the classical serotype 1, which was responsible for mortality in chickens in these outbreaks. All the isolates used in this study are identical.

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REFERENCES

- Anon. (1995). Epidemiological news, Department of Animal Production and Health. 5(4): 8-9.
- Hitchner, S.B. (1970). Infectivity of infectious bursal disease virus for embryonating eggs. *Poult. Sci.* 49: 511-516.
- Lukert, P.D. and Saif, Y.M. (1991). Infectious bursal Disease. pp. 648-663. *In: Calneck, B.W., Barnes H.J., Beard, C.W., Reid, W.M. and Yoder, H.W. Jr. (Eds). Diseases of poultry, Iowa State University Press, Iowa.*
- McNulty, M.S., Allan, G.M. and McFerran, J.B. (1979). Isolation of infectious bursal disease virus from turkeys. *Avian. Pathol.* 8: 205-212.

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Mekkes, D.R. and Dewit, J.J. (1994). Diagnostic tools in detection. World Pouly. Misset. Supplement. pp. 10-11.

Wyeth, P.J. and Chettle, N.J. (1988). An agar gel diffusion test for differentiating between strains of type 1 IBD virus. Vet. Res. 122: 442-443.