APPLICATION OF COUNTER IMMUNO ELECTROPHORESIS IN THE DETECTION OF ANTIBODIES TO INFECTIOUS BURSAL DISEASE OF POULTRY AND ITS USE IN HETEROLOGOUS DETECTIONS

K. Indumathi¹, N. Pushpa¹ and C. Anchana Devi²

Abstract

Counter Immuno Electrophoresis is a Sensitive immuno-diffusion technique used for the diagnosis of various diseases including viral diseases. Infectious bursal disease (IBDV) virus affects the bursa of fabricius of young chickens which results in mortality. The present study was designed to use this technique for the detection of antibodies against Infectious bursal disease of poultry and in the detection of Human immuno deficiency virus by using heterologous antigens. Antibodies required are prepared by propagating virus in embryonated chicken eggs using live vaccines.

Key Words: Infectious Bursal Disease Virus, (IBDV) Counter Immuno Electrophoresis (CIE), Human Immuno deficiency Virus (HIV), New castle Disease (ND).

INTRODUCTION

Antigen and antibody reactions are the basic principle in immunodiagnosis. Immuno rheophoresis (IR) is a technique used for the detection of Infectious bursal disease antigen from bursae collected from field cases and experimentally infected chicken (Raj et. al., 1998). Counter immuno electrophoresis is an immuno diffusion technique performed with the force of electric current. This is a sensitive test, can be performed quickly and it requires cheap accessory only. This technique is now widely used in the diagnosis of various infectious diseases especially viral diseases. Infectious bursal disease (IBD) is an acute highly contagious viral infection of young chickens that has lymphoid tissues as it’s primary target with a special predilection for the bursa of fabricius (Cosgrove, 1962). IBD is a member of Birnaviridae family (Brown 1986; Muller et. al., 1979) The viruses in this family are genomes consisting of two segments of double standard DNA (ds DNA) (Mac Donald, 1980). Infectious bursal disease virus (IBDV) affects the Bursa of fabricius results in morbidity, mortality and immuno suppression. Immuno suppression enhances the susceptibility of chickens to other infections and interferes with vaccination against other diseases. With this background the present project is designed to apply counter immuno electrophoresis for the diagnosis of IBDV and to study the possibility of using IBDV antigen for the purpose of detecting antibodies to Human immuno deficiency virus (HIV).

MATERIALS AND METHODS

The inoculums for the IBD virus cultivation is available as vaccines which
is manufactured by vankateshwara Hatcheries Pvt. Ltd., at Pune is obtained from Namakkal market. Nine (or) ten day old embryonated layer chicken eggs were obtained from M/s. Jaya devi hatcheries, Namakkal and checked for live embryo and eyespot. An air sac was marked and hole was made. Virus was inoculated in the hole, after incubation, allantoic membrane was broken and the fluid was collected. IBD specific rabbit hyper immune serum was obtained from Avian disease lab, Namakkal.

Known positive HIV serum was obtained from ELISA kit and suspected serum was obtained from Bharat Clinical Laboratory, Namakkal.

A clean dry glass slide was taken which was poured with agar. After solidification, eight wells were made in each glass slide. After the preparation of agarslide, Barbitone buffer was poured to the migration chamber, the slide was kept at the centre of the chamber, cathode and anode wires were connected to the power pack. 200 volts were set in power pack for 1 hour.

Agarslide was dried overnight in incubator and the dried slide was stained with coomassie blue for 10 minutes and destained with destaining solution.

Identification of the virus by direct immuno fluorescence staining, (McFerran. et al., 1980), nucleic acid probes, (Jackwood, 1988) and antigen capture enzyme immunoassay using monoclonal antibodies, (Snyder. et al., 1986) are some important diagnostic tools for detection of the viral antigen directly from the infected tissues.

RESULTS AND DISCUSSION

The serum used in the study is hyperimmuneserum raised in rabbit. Normal poultry serum served as negative control may contain unknown antibodies for other avian viruses which can not be ruled out completely. New castle disease (ND) specific serum, IB specific serum and normal rabbit serum are also treated with IBD antigen. From the analysis, it is evident that a clear sharp precipitation line is formed between IBD antigen and IBD serum. There is no precipitation line is obtained between IBD antigen and positive control serum for New Castle disease (ND), Infectious bronchitis (IB) and Normal rabbit serum. In the same way different types of antigens are treated with IBD serum, but precipitation line is obtained only between IBD antigen and serum and not with the other antigens. (Fig 1)

Fig. 1 : Counter Immuno eletrophoresis of prepared IBD positive antigen (1,3,5,7) with ND positive serum (2), IBD specific rabbit serum (4), IB positive serum (6), Normal rabbit serum negative (8)

The three serum samples suspected for HIV are subjected to CIE with IBD antigen and are found to yield no precipitation line. (Fig 2)
Fig. 2: Counter Immuno eletrophoresis of prepared IBD positive antigen (1,3,5,7) with IBD specific rabbit serum (2) and various HIV suspected serum samples (4,6 & 8)

HIV positive control serum and HIV negative control serum along with one normal human serum is subject to CIE with IBD antigen. HIV positive serum produced clear precipitation line and negative did not produce precipitation line. (Fig. 3).

Fig. 3: Counter Immuno eletrophoresis of prepared IBD positive antigen (1,3,5,7) with HIV suspected serum (2), HIV positive Elisa Kit control serum (4), HIV negative Elisa Kit control serum (6) and Normal human serum (8)

Failure of precipitation bands between IBD antigen and HIV suspected serum could probably be due to the advanced stage of the disease, because antibody detection in advanced cases may not be successful in 100% of the cases, (Patella et al., 1998). On oral enquiry made with the clinical laboratory, which supplied the HIV suspected serum revealed that the detection by ELISA with those samples also showed negative only. False negative results of most of the serological test with sera samples from advanced stage of patients checked with blind fold social barriers are the daunting problems of HIV control in India leading on to silent spread. (Chreb, 1997).

REFERENCES

