FLUORESCENT ANTIBODY TECHNIQUE IN THE DIAGNOSIS OF RINDERPEST

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INTRODUCTION

Rinderpest, a dreadful viral disease affecting principally bovines besides other species of domestic and wild animals, has been a subject matter of extensive and intensive research for the past several decades. Besides the clinical symptoms and postmortem lesions, biological test in susceptible calves has been the test of choice in diagnosing Rinderpest antigen. (Scott and Brown, 1961) Recently isolation of the virus in tissue culture systems is being attempted increasingly. Many serological tests are available to monitor the antibodies (Plowright and Fenris. 1957; Nakamura and Wagatuma, 1940; Ramachandran and Scott, 1972; Provost et al 1964; Sankaralingam et al 1979). There are few tests to detect Rinderpest antigen. Evaluation of each and every technique has not been carried out. The present paper records the results of the study in identifying Rinderpest antigen in lymph nodes with the help of fluorescent antibody technique (FAT) and evaluates the test.

MATERIALS AND METHODS

Lymph nodes:

Lymph nodes of goats used for the production of Rinderpest Goat tissue vaccine at the Institute of Veterinary Preventive Medicine, Ranipet formed the test lymph nodes. Mesenteric and prescapular lymph nodes were collected aseptically and carried over ice. They were preserved at —20°C until use. Lymph nodes from apparently healthy goats slaughtered at the Corporation slaughter house were used as negative control and Lymph nodes from known positive cases proved positive by more than one technique formed the positive control.

Rinderpest hyperimmune serum

Cross-bred calves (about one year old) were immunised with 10 doses of tissue culture rinderpest vaccine (TCRV). Subsequently

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they were given four weekly booster injections each consisting of 10 doses of Goat tissue vaccine (GTV). Antibody titre was tested by measles haemagglutination inhibition test.

The animals were bled on 35th day, serum separated and stored at -20°C until use

Separation of immunoglobulins

Immunoglobulins were separated by ammonium sulphate precipitation technique following in general the methods of Kawamura (1977). Protein estimation of the precipitated antibody globulins was done by micro-kjeldhal method and its purity was checked by agar gel electrophoresis. The globulin fraction moved as a single band.

Conjugation of immunoglobulins with Fluorescein isothiocyanate (FITC)

Modified Kawamura's method (1977) was used. Gamma globulin was adjusted to 2% concentration in NSS 0.8 mg of FITC (International Biological Suppliers Inc.) corresponding to 1/200 to 1/250 to the amount of protein was dissolved in 0.8 ml of 0.5M carbonate buffer pH 9.5. FITC solution was prepared just before use and added dropwise to globulin solution with constant stirring 20 to 25°C for 2 hours. Free dye was removed by passing through a Sephadex G 25 column.

Removal of the free dye

To avoid proceeding of the conjugation reactions beyond optimum level, step I and step II of Kawamura (1977) were carried out in succession. Sephadex G50 Columns were prepared (20 cm x 2 cm) and washed with two or three volumes of 0.005 M phosphate buffer pH 7.2 containing 0.1M sodium chloride. Two ml of the conjugate was layered on top of the bed and fractionated using effluent buffer. Samples were collected in 1 ml aliquots. The absorptions at 280 nm and 495 nm of each sample were measured in a Zeiss UV spectrophotometer and the fluorescein/protein molar ratio (F/P) and optical density (OD) ratio were calculated as per the methods of The and Feltkamp (1970) by using the following formula.

Amount of protein can be determined at 280 nm and dye at 495 nm. Molecular F/P ratio was calculated assuming a molecular weight of 160,000 for IgG and 390 for FITC.

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\text{Mol F/P ratio} = \frac{2.87 \times E^1 495}{E^1 280 - (0.35 \times E^1 495)}
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\text{OD ratio} = \frac{2.37 + (0.35 \times \text{Mol F/P ratio})}{\text{Mol F/P ratio}}
\]
The fractions having OD ratios between 0.5 to 1 were used for further purification procedures as per Johnson and Dorling (1977).

Purification of labelled antibody

Chromatographic purification with cellulose ion exchangers was carried out at 4°C as per Holborow (1970) Diethyl amino ethyl cellulose (DEAE cellulose—What No. II) was prepared as described by Peterson and Sobert (1962) and Himmelhoch (1971).

To 10 volumes of buffer (0.005M phosphate buffer with 0.1M sodium chloride pH 7.2) one volume of washed adsorbent was added and packed in the column. The column was allowed to equilibrate with 2 to 3 columns of buffer. Column was drained, 2 ml of the sample was applied to the column. When the sample has entered into the column three volumes of the following buffers were applied.

1. 0.005M Phosphate buffer (PB) containing 0.005M NaCl pH 7.2
2. 0.005M PB containing 0.05M NaCl, pH 7.2
3. 0.005M PB containing 0.5M NaCl, pH 7.2.

Fractions were collected in one ml aliquots, the OD was measured at 495 nm and 280 nm and OD ratio was calculated. Those tubes having a OD ratio of one was used for fluorescent staining.

Staining procedure

Glass slides were washed thoroughly in neutral cleaner soaked in ethanol containing 3% hydrochloric acid dried over a bunsen flame.

The lymph nodes were allowed to thaw to room temperature. A small cut was made with a sharp knife and the slides were pressed against fresh surface of the lymph node to prepare impression smears. The preparation was air dried and fixed in precooled acetone at -20°C for 10 minutes.

The smears were then washed with phosphate buffer saline (PBS) pH 7.2 thoroughly, and layered with, few drops of conjugated anti-rinderpest bovine immunoglobulin and incubated in a humid chamber for one and half hours at 37°C. They were then thoroughly washed in three changes of PBS for 10 minutes each, and air dried and mounted in mounting medium (1 part of carbonate buffer + 9 part of glycerol) and examined under Olympus fluorescence microscope using BG 12 as excitor filter and OG 1 as barrier filter.
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Fig. 1

Depicts the positive fluorescence emitted by cells which contain the Rinderpest antigen
RESULTS AND DISCUSSION

Out of the 95 lymphnodes from goats sacrificed for the preparation of rinderpest goat tissue vaccine, 85 lymphnodes proved to be positive and 10 lymphnodes proved to be negative with a probability percentage of 89.47% ± 9.42%. The Probability Percent was calculated according to Snedecor and Cochran (1967). Figure 1 depicts the positive fluorescence emitted by the cells which contain the rinderpest antigen.

FAT has proved its usefulness in the study of development of rinderpest virus in cells in vitro but its application as a diagnostic aid was not successful so far because of the problem of background fluorescence (Leiss and Flowright, 1963). In the present study background fluorescence could be removed because of the purification of the conjugate by chromatographic method. Specific fluorescence could be observed over the surface of the lymphoid cells as well as in the cytoplasm.

In the present study since impression smears were used such clear delineation could not be observed. Prabudas and Sambamurthi (1976) detected rinderpest antigen by indirect FAT in bovine kidney cell cultures. Since the lymph nodes tested were from goats showing reaction to rinderpest virus, all the lymphnodes should have presented positive fluorescence. But only 89.47% per cent of lymph nodes were positive and yielded a confidence level of 89.47% ± 9.42% though these lymphnodes proved positive by other serological tests. It is not clear as to why 10 lymphnodes which were positive by other serological tests were negative by FAT and requires further study.

FAT can therefore be used for diagnosing rinderpest antigen along with another serological test so that the false negatives could be detected. FAT is particularly useful because of being quick specific test.

SUMMARY

A Procedure for conjugation of FITC to globulins and FAT is described. FAT was used for detecting Rinderpest antigen in impression smears of lymphnodes from goats employed for the production of Rinderpest Goat tissue vaccine. FAT could detect Rinderpest antigen in 89.47 ± 9.42 per cent of the cases.

REFERENCES


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