Summary of PhD thesis (1987)

Address: (Comparative studies on the diagnostic tests used for rinderpest)

In this study some of the recently applied serological techniques, had been developed and evaluated for the detection of RP- viral antigens and antibodies like dot – ELISA, solid phase ELISA, cell ELISA, and staphylococcus protein A agglutination test (SPA). These techniques had been compared with the traditional techniques like agar gel precipitation test and florescent antibody technique that previously used for the diagnosis of RP.

Different methodological variable as temperature of incubation, time of incubation and type of diluents had been adopted for the preparation of agar gel precipitating antigens.

In this study, it has been shown that RP- viral strain , which was grown on Vero cells for 96 hours and extracted using N.B.40 solvent, is the strongest if used in the AGPT (it gave a clear precipitating line after 24 hours) and the Dot ELISA (where it gave strong blue dots 3 hours after the start of the test even with positive serum dilution 1:25 till 1: 200) although it is preferred to use the antigens prepared in the bacterial SPA agglutination test as it proved its preference for the Dot ELISA antigen prepared by using N.B.40, and it gave a positive result two minutes later with the presence of staphylococcus already adsorbed with positive serum diluted 1:10 and carried on its surface IgG antiviral bodies. The bacterial SPA test was used to determine the virus antigens using a microscopic slide and the bacterial aggregations were seen within 2-minute by the naked eye and it became very clearly as it was filmed from under the light microscope, and this method was the best and fastest way to diagnose bovine plague as the disease can be diagnosed in two minutes It had been proved that the NP-40 cell extracted viral antigens is potent and more reliable for the detection of RP- antibodies in bovine sera. Staphylococcus protein A agglutination test (SPA) was used as slide and micro plate assay and found to be easier, rapid and more sensitive than the AGPT. Parallel to the above mentioned, the Dot – ELISA had been proved to be the most sensitive and versatile techniques in detecting the RP- viral antigen in infected tissue culture, organs and secretion of naturally infected animals. By the IFA, the cytoplasm inclusion bodies of virus-infected Vero cells was seen from 12 hours after injection, then more widespread and visible in cytoplasm and cell nuclei after 72-96 hours of injection into cells. When identifying the bovine plague virus in the organs and secretions of animals infected with the virus by using the above-mentioned tests, it became clear that the dot-Eliza test was the best of these tests, followed by the bacterial SPA test and the AGPT, where the percentage of positive samples was 82.6%, 65.2% and then 17.3%, respectively.

The best methods and fixation of cells on plastic 96 micro titer plate using acetone, absolute ethanol, absolute methanol, a mixture of ethanol and methanol in 50% , acetone with methanol 50%, acetone with ethanol by 50%, acetone with ethanol and methanol by 60%, 25%, 15% respectively and the best method turned out to be the last

As for the identification of anti-bovine plague immune bodies in sera, both the VN test, the solid phase ELISA, cellular ELISA and the bacterial SPA test were used to determine the titers of immune bodies in the sera used. The tests were first evaluated using a hyper immune serum of antibodies prepared against the bovine plague virus in rabbits with both the antigens prepared, beside they were performed on 100 samples of cow serum (66), buffalo (15), sheep (2), goats (15) and horses (2).Rinderpest antibodies could be detected in rabbit and bovine sera using the VNT, AGPT and the recently used SPA – agglutination test, Dot ELISA, solid phase ELISA and cell ELISA. The obtained results showed clearly that the newly introduced SPA agglutination test and

dot ELISA proved to be high sensitive techniques which could be used in combination with the other conventional serological techniques. These techniques proved to be conventional and reliable for the diagnosis of RP. It is of importance to mention that, the development of the micro titer plate SPA agglutination test triggers the introduction of this new screening system for RP – antigens and antibodies and helps in the rapid diagnosis of RP in field samples as compared with the SNT and AGPT. It can also be concluded that, it is possible to use solid phase ELISA plates already coated with measles antigens for the detection of rinderpest antibodies in bovine serum samples.