Indian Farmer
Volume 9, Issue 09, 2022, Pp. 410-412.
Available online at: www.indianfarmer.net
ISSN: 2394-1227 (Online)

ORIGINAL PAPER

Role of Enzyme Linked Immunosorbent Assays in Effective Implementation of Foot-and-Mouth Disease Control Programmes going on in India for the past two decades

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Article Received: 19 September 2022 *Published Date: 23 September 2022*

Foot-and-mouth disease (FMD) is one of the most important viral diseases occurring worldwide and causing huge economic losses. It is a highly contagious disease of cloven-hoofed animals including cattle, buffalo, goat, sheep, pig and wild animals. Out of all the species affected, cattle and pigs are the more severely affected. It is caused by FMD virus (FMDV), a member of the genus *Aphthovirus* belonging to the family *Picornaviridae*. The virus has four structural proteins and eight non-structural proteins. The FMDV has been grouped in seven immunologically distinct serotypes: O, A, Asia-1, C, South African Territories (SAT)-1, 2, and 3 across the world. In India, FMDV serotypes O, A and Asia-1 are most prevalent and serotype C has not been reported since 1995.

The incubation period for the disease ranges from 2 to 14 days. In cattle, the disease is characterized by fever, decrease in feed intake, decline in milk production, profuse salivation, smacking of lips, lameness and vesicle formation on the tongue, interdigital space of foot, udder and teats. In pigs, the vesicle formation occurs on the feet or snout and lameness is the most prominent sign. Mild symptoms of fever and lameness can also be observed in sheep, goats and wild ruminants. FMD results in high morbidity and low mortality, however the mortality rates can be high in young animals when virus invades their heart muscles. The disease has been proved to be a serious threat to the economy of the farmers and the country. It results in decrease in milk yield, working capacity and overall growth in the affected animals which forces the helpless farmers to spend a significant amount of money for veterinary services and medicine to control the disease. Therefore, it is very important to develop strategies to control the diseases.

In the 2003-04, the Government of India (GoI) initiated FMD Control Programme (FMD-CP) with an aim to control and eradicate the disease by mass vaccination of susceptible population of cattle and buffaloes at six months interval. It was initially

launched in 54 districts covering eight states and five union territories (UT) across the country. At present, the programme has been expanded over whole of the country in a phased manner. In 2019, National Animal Disease Control Programme (NADCP) was launched with funding from Department of Animal Husbandry and Dairying, GoI to vaccinate 100% of the susceptible population of cattle, buffalo, sheep, goat and pig against FMDV with an aim to control the disease by 2025 and its eventual eradication by 2030. Earlier, under FMD-CP for FMD sero-monitoring, pre- and post-vaccination serum samples were collected from 10 cattle and 10 buffaloes randomly from 10 villages of each district in the State. The serum samples were screened for the presence of FMDV serotype O, A and Asia-1 specific neutralizing antibodies using enzyme linked immunosorbent essay (ELISA).

ELISA is an immunological assay that is commonly used to detect and quantify an antigen, antibody or protein in a biological sample. It is performed in a 96 well polystyrene microtiter plate. It exists in different forms – direct, indirect, competitive, sandwich, etc. Generally, in ELISA an antigen or antibody is coated on the plate. The sample containing antigen or antibody in appropriate dilutions is added later. Subsequently, there is formation of an antigen-antibody complex that is detected by an enzyme, linked to an antibody (primary or secondary). A substrate is added, upon which the enzyme reacts to form a coloured compound. In the last step the reaction is stopped using a stopper solution. After every step, the plates are incubated for an appropriate time-temperature combination and washed using washing buffer that removes the extra unbound compound from the plate. The inference from ELISA test can be made in three ways. Firstly, qualitative estimation in which the presence or absence of an antigen or antibody is detected on the basis of absence or presence of colour development. Secondly, semi-quantitatively, where the amount of antigen or antibody is linked to the intensity of colour development. Lastly, the quantitative method, where the amount of antigen or antibody in a biological sample is estimated on the basis of a graph obtained by plotting the different optical density (OD) of a standard solution at different known concentrations. The optical density is measured using spectrophotometer at appropriate wavelength on the basis of the type of enzyme and substrate used in the test.

Different types of ELISA tests are used in the sero-monitoring and sero-surveillance of FMD in India, that are developed by the Scientists of ICAR-Directorate of FMD (ICAR-DFMD). Initially, liquid phase blocking ELISA was used for pre- and post-vaccination antibody monitoring against FMDV, which has been replaced by solid phase competitive ELISA (SPCE) since 2017-18. The SPCE is based on the competition between FMDV serotype specific antibodies in the test sample and FMDV serotype specific tracing antibodies, where the FMDV antigen was blocked by FMDV serotype specific antibodies in test samples (if present), which further prevented the binding of tracing antibodies to FMDV antigen. Apart from demonstrating vaccination efficacy, these ELISAs are also prescribed by the World Organization for Animal Health (OIE) for international trade i.e., to certify individual animals prior to import or export.

Vaccination is the principal strategy followed to control and eradicate the FMD in countries like India where test and slaughter policy cannot be followed. At present, inactivated FMD vaccines or combined vaccines are available that are devoid of non-structural proteins (NSPs) of FMDV and contains only structural proteins. This formed the basis for the differentiation of vaccinated from FMD infected animals (DIVA), which is being done using NSP- ELISA. The naturally infected animals show antibodies against both structural and non-structural proteins, whereas vaccinated animals formed antibodies only against structural proteins. Different NSP ELISA for FMDV are available based on 2B, 2C, 3A, 3AB, 3B, 3ABC and 3D NSPs. Of these, the 3AB3 NSP-ELISA is presently used in the country. Additionally, for FMDV detection, a sandwich ELISA is performed to serotype the clinical material, in which the serotype specific antigen is sandwiched between serotype specific coating and tracing antibody raised in different experimental animals. Presently research is going on in the department of the authors to replace these antisera with synthetic or recombinant antibodies thus promoting animal welfare.

The ELISA test has many advantages over other diagnostic test making it a popular option. The test has high sensitivity, specificity, efficiency and is able to detect antigens at the picogram level. It is a simple procedure and is easy to perform. It is a cost-effective assay, has high throughput and can be used to test various sample types like serum, urine, milk, etc. It is both qualitative and quantitative test. Many samples can be tested simultaneously on a single microtiter plate. It is a safe test and has no negative impact on the environment as no radioactive or other harmful reagent or by-products are released or involved. These points justify the importance and use of ELISA test over other tests making it a wise and prime choice that is practiced countrywide for FMD sero-surveillance and for monitoring the effectiveness of the vaccination control programme.