Application of competitive ELISA in the diagnosis of equine infectious anaemia

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A competitive enzyme-linked immunosorbent assay (CELISA) was used and compared with the internationally recognized Coggins's test for the sero diagnosis of equine infectious anaemia (EIA), a dreaded disease of equines, using a kit consisting of enzyme conjugated group specific p26 antigen and monoclonal antibody to it. Parallel testing of sera samples from EIA suspected and in-contact horses indicated that both the tests detected identical positive reactors. The correlation between the two tests was greater than 99 per cent.

Equine infectious anaemia (EIA), a viral disease of equidae, is caused by a lentivirus of family Retroviridae to which human AIDS virus HIV also belongs. EIA in horses is characterized by intermittent fever, anaemia, oedema of dependent parts, periodic relapses and persistent infection. In India, EIA was first confirmed clinically and serologically by Uppal and Yadav\(^1\) in Thoroughbred horses from Karnataka.

The serological test used for the diagnosis of EIA is agar gel immunodiffusion test known as Coggins's test. In the United States, this test is approved by the US Department of Agriculture and is conducted in the approved laboratories of USA in 49 States\(^2\). These laboratories are said to be selected on the basis of competence, integrity and too much cooperation in governmental programme. The Coggins's test performed to confirm EIA in India by us was based on the method which is also prescribed in the \textit{OIE International Animal Health Code} (5th edn., pp. 323–327) and in the \textit{OIE Manual of Recommended Diagnostic Techniques and Requirements for Biological Products} (vols. 1, 16). Interpretation of results of Coggins's test was based on the international reference serum which represent the minimum level of detection to be achieved by the laboratory using Coggins's test.

However, in the recent past, enzyme linked immunosorbent assay (ELISA) test has been standardized by Shen \textit{et al.}\(^3\) and Archambault \textit{et al.}\(^4\). But this test is not yet accepted as an official test in any of the European countries, including the United Kingdom.

In the present studies, 16 sera of intermittent fever cases and 10 sera of in-contact animals in the same vicinity attended by the same clinician attendents were selected for performing Coggins's and ELISA tests. In the initial testing by Coggins's test, only 15 out of 16 sera were sero-positive. The remaining 1 serum from fever case and 10 sera of in-contact animals were seronegative. All the 26 sera were subjected to competitive ELISA (CELISA) using a kit (Dia systems CELISA EIA Antibody Test Kit). The kit consists of purified EIA antigen and monoclonal antibodies to the group specific p26 antigen. The test in brief consists of the incubation of test serum simultaneously with the enzyme conjugated p26 antigen in the plastic wells precoated with monoclonal antibody specific to p26 antigen. Antibodies to EIAV if present in the test serum competes with the bound anti-p26 monoclonal antibodies for the enzyme-linked purified p26 antigen. Little or no colour development as a result of the prevention of the binding of the enzyme-linked antigen to the monoclonal antibody by the EIAV antibodies present in the test serum, indicates positive test result which is compared with positive and negative control sera. The colour change of the substrate solution to blue green indicates the absence of EIA-specific antibodies in the test serum.

After addition of 0.10 ml each of test serum sample and 0.05 ml of antigen conjugate into wells precoated with monoclonal antibody and thorough mixing, these were incubated 30 min at 37°C before washing; addition of substrate solutions and taking readings as described in the directions supplied along with the kit.

The results obtained showed that all the 15 Coggins's test positive sera samples were also positive by CELISA while the horse sera which were negative to Coggins's test were also negative by CELISA. One of the horses which showed fever could not be diagnosed as EIA by either of the 2 tests, responded to the broad spectrum antibiotics and other supportive treatment and did well in work performance compared to the EIA-positive horses which did not respond to treatment and showed poor work performance and periodic relapses of fever. The correlation thus obtained with Coggins's and CELISA was as expected, i.e. greater than 99 per cent for the kit used.

The present observation confirms that ELISA test can be used for detecting the antibodies to the main group-specific antigen of EIAV which is present in the virion core—the p26 antigen\(^5\), which is also detected by Coggins's test. On the basis of these findings, efforts are now being directed indigenously to develop the ELISA test using the p26 antigen which is available from Tech America so that the large number of samples in India could be tested. However, it remains to be seen whether the European countries as well as the Government of India, Ministry of Agriculture, accept this more sensitive test as a singleton confirmatory test as the interest of horse owners cannot be underestimated because of economic damages. Nevertheless, the standardization and development of ELISA test in India will be useful for the researches on EIA.

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