ENZYME-LINKED IMMUNOSORBENT ASSAY OF SERUM ANTIBODIES TO MAREK'S DISEASE VIRUS IN VACCINATED CHICKENS

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We used an enzyme-linked immunosorbent microassay (micro-ELISA) to detect serum antibodies acting with Marek's disease virus (MDV) in chickens vaccinated with apathogenic turkey herpesvirus (HVT). The micro-ELISA (*1*) was carried out in flat-bottom microtiter plates (Dynatech, Switzerland) and the presence of antibodies reacting with MDV was evaluated visually based on the colour change. Agar gel precipitation (AGP) and radial immunodiffusion (RID) test (*2, 3*) were performed in parallel.

We tested 106 sera collected at random from commercial HVT-vaccinated chickens. Sera from non-HVT-vaccinated and MDV-free birds served as controls. Feather follicles of each bird were assayed in RID tests. Hyperimmune serum against MDV was prepared (*4*) and stored at −20° C. MDV antigen from feather follicles (*5*) was concentrated and purified. Control feather follicle antigen from unvaccinated MDV-free birds was prepared similarly. Rabbiet anti-chicken IgG was conjugated with horse-radish peroxidase (Type VI, Sigma, St. Louis) by a two-step method (*6*) and stored at 4 °C for one week; it was used in a 1 : 40 dilution which gave a disrinct reaction. Known MDV-negative and -positive antigens, known MDV-negative and -positiv antisera were included in control wells besides a blank control.

Micro-ELISA detected antibodies cross-reacting with MDV in all serum samples, while the AGP test detected them only in 43.4% of sera; 3.8% of the chickens shed virus in feather follicles. All controls were negative (numerator: No. positive; denominator: No. tested; in parentheses: % positivity):

Flock, age	Sera		Feather follicles
	Micro-ELISA	AGP test	RID test
A, 3 months	39/39 (100)	9/39 (23.1)	2/39 (5.1)
B, 6 months	25/25 (100)	10/25 (40.0)	1/25 (4.0)
C, 6 months	10/10 (100)	4/10 (40.0)	1/10 (10.0)
D, 13 months	10/10 (100)	3/10 (30.0)	0/10 (0)
E, 15 months	22/22 (100)	20/22 (90.9)	0/22 (0)
Total	106/106 (100)	46/106 (43.4)	4/106 (3.8)

The sensitivity of the technique can be judged by the fact that it detected antibodies cross-reacting with MDV in all sera from 3 months old chickens as compared with 23.1% of positive sera detected by the AGP test. The RID test revealed that 3 of 4 birds shedding virus had cross-reacting antibodies in their blood, supporting the hypothesis that active antibody production has no significant effect on protection from MDV infection. The micro-ELISA described here may be recommended for epidemiological surveys and determination of the serological status of vaccinated chickens.

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