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Diagnosis of recent and late *toxoplasma gondii* strain rh by two-dimensional immunoblot of chicken immunoglobulin g and m profiles

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Toxoplasma gondii is an intracellular apicomplexan parasite that infects a wide range of warm -blooded vertebrate hosts. The protein expression of *T. gondii* was determined by image analysis of the tachyzoites proteome separated by standard-one and conventional two-dimentional gel polyacrylamide electrophoresis (2D- PAGE). The macro- and micro- scale gels of the tachyzoite proteome were stained with Coomassie brilliant blue and the high sensitive Silver stain. Pooled gels were prepared from tachyzoites of *T. gondii*. A representative gel spanning a pH range of 3-10 of the tachyzoite proteome consisted of 1306 distinct polypeptide spots. Two-dimensional electrophoresis (2-DE) combined with 2-DE immunoblotting was used to resolve and compare immunoglobulins (Igs) M & G patterns against Toxoplasma gondii strain RH (mouse virulent strain). The subsequent transfer of the resolved polypeptide spots of the tachyzoite proteome to immobilon®-P transfer membrane has been done using electrophoretic blotting. The broader picture of immunologically reactive polypeptide spots of *T. gondii* has been revealed by IgM and IgG *Toxoplasma* antibodies from SPF chickens that were subcutaneously infected with $5x10^6$ live tachyzoites of *T. gondii* strain RH at different times. Total tachyzoite proteins of *T. gondii* were separated by two-dimensional gel electrophoresis and analyzed by Western blotting for their reactivity with the 7 and 56 days post-infection (dpi) SPF chicken antisera. Relying upon immunoglobulin M and G, different antigenic determinant patterns were detected. Of the total number of polypeptide spots analyzed (1306 differentially expressed protein spots), 6.97% were detected and identified as shared antigenic polypeptide spots on immunoblot profile using IgG and IgM antibodies regardless the time after infection. Furthermore, some of the immunoreactive polypeptide spots seemed to be related to the stage of infection. Interestingly, we reported the presence of natural antibodies to toxoplasmic antigens, in addition to the highly common conserved antigenic determinants that reacted with non-specific secondary antibody; goat anti-chicken IgG (H+L chain specific) antibodies conjugated with horseradish peroxidase. In conclusion, unique reactive polypeptide spots are promising candidates for designation of molecular markers to discriminate recent and late chicken infection.

Biography

Saeed Elashram is a professor of parasitology at Faculty of Science Kafr El-Sheikh University. He has published so many papers in reputed journals. He had four registered patents and contributed in many international conferences.

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