SUMMARY

Toxoplasmosis caused by the intracellular parasite *Toxoplasma gondii* is a zoonotic disease of worldwide distribution. During pregnancy, *Toxoplasma* infection may cause spontaneous abortion, stillbirth or premature delivery in addition to various congenital anomalies depending on the gestational age and strain type.

Although only one species of the genus *Toxoplasma* has been known, biological studies have suggested the presence of three strains (I, II, III) that differ in their virulence in mice. Type I is highly virulent for mice producing acute toxoplasmosis while type II and III are less virulent, producing chronic infections in mice.

The aim of present study is: isolation, biological typing and characterization of local Egyptian *Toxoplasma* isolates from complicated pregnant cases in addition to detection of *Toxoplasma* infection by microscopical, serological and molecular techniques.

The present study was carried out on 106 women presenting with complicated pregnancy classified in four groups. The first group included 56 women with abortion in the first trimester, the second group included 30 women with abortion in the second trimester, the third group included 15 women with intrauterine fetal death while the fourth group was deliverers of babies with congenital anomalies and included 5 cases.

From all cases included in the study, serum and corresponding tissues samples were collected. Sera of all cases were tested using latex agglutination test and enzyme linked immunosorbent technique for detection of *Toxoplasma* antibodies. Each tissue sample was digested,

microscopically examined and inoculated individually in three mice if suspected to contain *Toxoplasma* bradyzoites.

Inoculated mice were followed up daily for the appearance of clinical signs of toxoplasmosis and then killed on 45^{th} – 60^{th} day post inoculation for detection of tissue cysts in their brains. Biological diversity of virulent isolates was detected by mice manifestations and tissue cyst formation.

Molecular assay using PCR was done for all tissue samples and for mice brain of the succeeded isolates to confirm that tachyzoites previously isolated from them belongs to *T. gondii* strain. SDS-PAGE was used for separation of protein components of RH strain and local isolates followed by identification of the immunoreactive components by immunoblot assay. Western blot was used as "reference test" for evaluation of the diagnostic accuracy of latex agglutination test, ELISA and PCR.

The study revealed that 36 (34%) of the examined tissue samples by microscopy were positive for the presence of bradyzoites. Only seven isolates (6.6%) succeeded to pass in mice where tissue cysts were detected in their brain and were confirmed by PCR detection. Biological typing of the seven isolates indicated the nonexistence of type I. They were all belonged to either type II or III (cystogenic strains).

Serological screening of the studied cases showed that 38 (35.8%) of the women were positive using latex agglutination test versus 45 (42.4%) cases by ELISA. 20 (18.9%) and 38 (35.8%) cases were seropositive for IgM and IgG antibodies respectively while 35 (33%) cases were positive by PCR.

Electrophoresis analysis of *Toxoplasma* antigens showed 15 bands for RH strain ranging in molecular weight from 116-17 kDa versus 11 bands ranging from 114-22 kDa for local strain. Immunoblot analysis showed five immunoreactive bands for *Toxoplasma* IgG (72, 66, 38, 30 and 26 kDa) versus three reactive bands for *Toxoplasma* IgM (24, 35 and 45 kDa).

Considering western blot as reference standard, the relative sensitivity was 82.5, 97.5 and 87.5 % while the specificity was 90.9, 92.4 and 96.9 % for latex agglutination, ELISA and PCR respectively. In the present study and according to the history taken from examined women, direct contact with house cats was not a risk factor while eating undercooked meat and contact with soil was a possible risk factor for *Toxoplasma* infection.