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SHORT COMMUNICATION

Seroprevalence of toxoplasmosis in voluntary blood donors of Puducherry and surrounding districts of Tamil Nadu

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Abstract Our objective is to study the seroprevalence of toxoplasmosis in the voluntary blood donors of Puducherry and surrounding districts of Tamil Nadu. A total of 275 healthy blood donors were screened for the presence of IgM and IgG antibodies to *Toxoplasma gondii* by ELISA test. Donor samples positive for IgM and/or IgG antibodies to *T. gondii* were subjected to IgG avidity ELISA. While, 54 out of 275 donors had IgG antibodies (19.66%), only one donor had IgM (0.36%) along with IgG. Among 54 IgG positive donors, only two had low avidity (3.7%), indicating recent exposure to the protozoa. Feasibility and cost effectiveness studies should be conducted throughout India to decide regarding screening of blood donors for toxoplasmosis.

Keywords Avidity · Blood donors · IgM ELISA · IgG ELISA · *Toxoplasma gondii*

Introduction

Toxoplasmosis is one of the most common parasitic infections, to which almost one-third of humanity is exposed to (Dubey 2005). Infection is transmitted to humans by their intimate association with domestic pets, especially the cats and consumption of uncooked/partially cooked livestock meat or drinking water contaminated with the cyst (Dubey 2005; Parija 2013). On the global level, Pappas et al. (2009), observed seroprevalence of 40–70% in South American and Caribbean countries. El Salvador, Germany and France show 75% seropositivity (Dubey 2005). Indonesia (Terazawa et al. 2003) and Ivory Coast (Adou-Bryn et al. 2004) has 60% prevalence. In the indigenous tribes of Malaysia, Ngui et al. (2011) recorded 37% seropositivity. Saudi Arabia (Al-Qurashi et al. 2001) and Qatar (Abu-Madi et al. 2010) has an equal prevalence of 25%. Jones et al. (2001, 2007) observed the decline in seroprevalence of general population of USA from 22% in 2001 to 11% in 2007. In France, there has been a decline from 55 to 37% in adults (Centre National de Référence de la Toxoplasmose 2013). World-wide, there are only a few reports of voluntary blood donors with *T. gondii* antibody positivity. A high 79% (in males) and 63.4% (in females) seroprevalence was seen in Brazilian donors (Coêlho et al. 2003) whereas a moderate 24.2 and 20.25% and a low 7.4% were observed in Slovakia (Studenicová et al. 2006), Turkey (Yazar et al. 2006) and Mexico (Alvarado-Esquivel et al. 2007) respectively. In Africa, varying prevalence of 7–80% was observed which came down to 6.4% in South Africa (Kistiah et al. 2011). Namibia recorded a low 0.96% positivity (van der Colf et al. 2014). New Zealand reported 42.9% seroprevalence (Zarkovic et al. 2007). In China, prevalence ranged from 0.4 to 20.2% (Zhou et al. 2011). Laksemi et al. (2013) recorded higher prevalence of 35.9% in men and 63.9% in women of Bali (Indonesia). Karimi et al. (2016) observed that Iranian blood donors had IgG and IgM antibodies ranging from 12.3 to 52.8% and 0 to 5.47% respectively. El-Sayed et al., (2016) from Egypt observed 33.67% prevalence. Chiang et al. (2012) from Taiwan found 9.3% positive for anti-Toxoplasma IgG, but

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only 0.28% positive for IgM. Makki and Abdel-Tawab (2010) from Saudi Arabia reported 40% positivity. From Thailand, Sukthana et al. (2001) observed 10% seropositivity. An exhaustive pan-India survey was conducted by Dhumne et al. (2007) involving 23,094 persons. The prevalence varied from 9.4% in Rajasthan to 48% in Kerala. Maharashtra and Goa had a positivity of 31–32%, followed by Tamil Nadu, AP, Karnataka and Kerala with 21.8, 22.2, 28.8 and 42.2% respectively. Bengal, Orissa, Assam, Tripura and Arunachal Pradesh had almost the same prevalence like south with 22–29%. Rajasthan, Punjab, Bihar, Delhi, UP and Chhattisgarh had a positivity varying from 9.4 to 19.9%. Another recent pan-India study (Singh et al. 2014) involved 1464 women of child bearing age. All India seroprevalence average was 22.4%, with lowest in Gujarat (8.8%), moderate in Delhi (19.7%) and Assam (21.2%) and highest in Manipal (37.3%). Since there is no report of toxoplasmosis from Puducherry for more than four decades, we have screened voluntary donors of Puducherry and surrounding districts of Tamil Nadu for this parasitic zoonosis.

Materials and methods

This analytical study has been carried out during the period of January to December, 2016 in the Departments of Microbiology and Pathology, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth University, Puducherry. Our Institutional Human Ethical Committee (IHEC) has approved this research project. Written Informed consent was obtained from the blood donors before collection of blood. Sample size calculation was made taking into account 20.3% prevalence in the neighbouring Karnataka State (Sundar et al. 2007). Specimens were collected in sterile tubes without anticoagulant, allowed to clot, centrifuged and serum was separated. Sera were aliquoted and kept frozen at −20 °C until the time of testing. The following three ELISA kits were used: (NovaTec Immunodiagnostica, GmbH, Dietzonbach, Germany)

1. NovaLisa *Toxoplasma gondii* IgG ELISA—TOXGO460
2. NovaLisa *T. gondii* IgM μ-capture ELISA—TOXMO460
3. NovaLisa *T. gondii* IgG Avidity Test—TOXGA460

Blood samples from 275 donors comprising 256 male and 19 female with age ranging from 18 to 65 years were screened for IgM and IgG antibodies to *T. gondii*. All positive samples were subjected to IgG avidity test. ELISA tests were carried out strictly in compliance with the procedure outlined in the kits' brochures. Patients' sera were initially diluted 1:100 with the sample diluent for all three ELISA tests.

IgM ELISA

IgM μ-capture ELISA wells are coated with anti-human IgM to bind with corresponding antibodies in the patients' samples. After incubation and washing, horseradish peroxidase (HRP) labeled *T. gondii* antigen was added and incubated further. Wells were washed and Tetramethylbenzidine (TMB) substrate added, incubated in dark and finally stop solution (Sulphuric acid) was added. Optical Density (OD) readings were taken at the wavelength of 450/620 nm in iMark Microplate Reader, Bio-Rad, Japan.

Cut-off value calculation was done as follows:

$$\text{Antibody index} = (\text{Sample O.D/Cut-off O.D}) \times 10.$$

IgG ELISA

The microtitre strips are precoated with inactivated *T. gondii* antigen. Serum samples were diluted 1:100 and added to the wells, incubated and washed. HRP labeled anti-human IgG conjugate was added, further incubated and plate washed. TMB substrate added followed by stop solution and OD readings were taken.

IgG avidity test

Procedure was almost similar to IgG ELISA with a slight modification, viz., the samples were run in duplicate, one well with avidity reagent (6% urea solution) and another well with wash buffer and IgG ELISA was performed as described.

Calculation of avidity results was done as follows:

$$\frac{\text{Absorbance (sample or control) Avidity reagent}}{\text{Absorbance (sample or control) Washing buffer}} \times 100 = \text{Avidity}\,(\%)$$

Interpretation of avidity results:

$$\text{Avidity values} > 40\,(\text{high avidity})\text{-Past infection}$$
$$\text{Avidity values} \leq 40\%\,(\text{low avidity})\text{-Acute or Recent infection}$$

Statistical analysis

Patients' data was analyzed by Chi square test, using GraphPad QuickCalcs (GraphPad Software Inc, USA) and p values of ≤ 0.05 were considered as statistically significant.

Results and discussion

Donors were aged between 18 and 65 years and mean age was 34.8 (SD 12.95 ± 12.97). Three out of 19 female and 51 out of 256 male, totaling 54 donors (19.66%) were

positive for *T. gondii* IgG antibody. Only one male donor was positive for IgM antibody (0.36%), who also had IgG. However, he was negative in the avidity test. Two male donors were positive in IgG avidity ELISA. There was no statistical difference between the two sexes as well as the three different age groups (18–34; 35–45; ≥46) regarding the seropositivity. The lone patient with both IgM and IgG antibody but with high IgG avidity could be placed in recent/active infection. A low anti- *T. gondii* IgG avidity index suggests recent infection within the past three to five months (Flori et al. 2008). Similar to our experience, Chiang et al. from Taiwan, reported that out of 1783 donors 166 (9.3%) tested positive for anti-Toxoplasma IgG, while 5 (0.28%) tested positive for anti-Toxoplasma IgM. All five IgM positive donors had high avidity antibodies suggestive of past infection.

Individual reports from different parts of India show a diverse picture: Highest 57% seropositivity in Kumaon (UP) (Singh and Nautiyal 1991) followed by Bombay 39% (Rawal 1959), Dibrugarh, Assam (24.3%) (Borkakoty et al. 2007), Jodhpur (17.2%) (Joshi et al. 1998), Chandigarh (5.4%) (Mohan et al. 2002) and Delhi (1%) (Mittal et al. 1990). Bhatia et al. (1974) from Pondicherry examined 80 but none was positive for *T. gondii* antibodies. Similarly, Shanmugam et al. (1995) could not get any seropositivity among 30 blood donors from Trivandrum. Nagarathna et al. (2013) from Bangalore, Karnataka examined 90 donors and found 23.33% positivity. Sundar et al. (2007) from Bangalore recorded *T. gondii* IgG 20.3% and IgM 3.6% seroprevalence among voluntary blood donors. Only 7% of them showed low avidity and 63% high avidity. Meisheri et al. (1997) from Bombay reported 30.9% seropositivity. Anti-Toxoplasma IgM was negative in all donors. Elhence et al. (2010) recorded anti-Toxoplasma IgG 51.8% and IgM 5%. However, IgG avidity was not carried out by the above two sets of authors.

Montoya (2002) considers that positive IgM result in an isolated sample can be interpreted as a true positive in acute infection, a true positive in chronic infection or as a false-positive. Ferreira and Camargo 2002 recommends that IgM test should be performed by a procedure with minimal nonspecific reaction, such as IgM-capture EIA. We have used NovaLisa *T. gondii* IgM μ-capture ELISA kit in our study. According to Reis et al. (2006), low IgG avidity in pregnant mother is a risk for fetal infection. The algorithm for serodiagnosis of toxoplasmosis in the USA includes an IgM measurement when time of infection needs to be defined. If IgM is positive at elevated indexes, infection has probably occurred in the prior three to six months. If it is positive at low indexes it may be a false positive, be related to infection acquired in the prior 2 years or be related to re-infection (Wilson et al. 1997). Antibody positive donors need not necessarily harbor the parasite in their blood. Parasitaemia could be detected by molecular diagnostic methods like PCR (Bastien 2002; Rahumatullah et al. 2012; Hallur et al. 2015). Donor blood with recent or active toxoplasmosis may pass on the infection to the recipients. The patients at high risk to contract toxoplasmosis after blood transfusion include recipients of organ transplants like kidney, heart and liver (Martina et al. 2011). To conclude it may be said that that more number of samples of blood donors need to be evaluated to finally conclude whether to include toxoplasmosis screening of blood donors or not.

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Compliance with ethical standards

Conflict of interest The authors declares that they have no conflict of interest.

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